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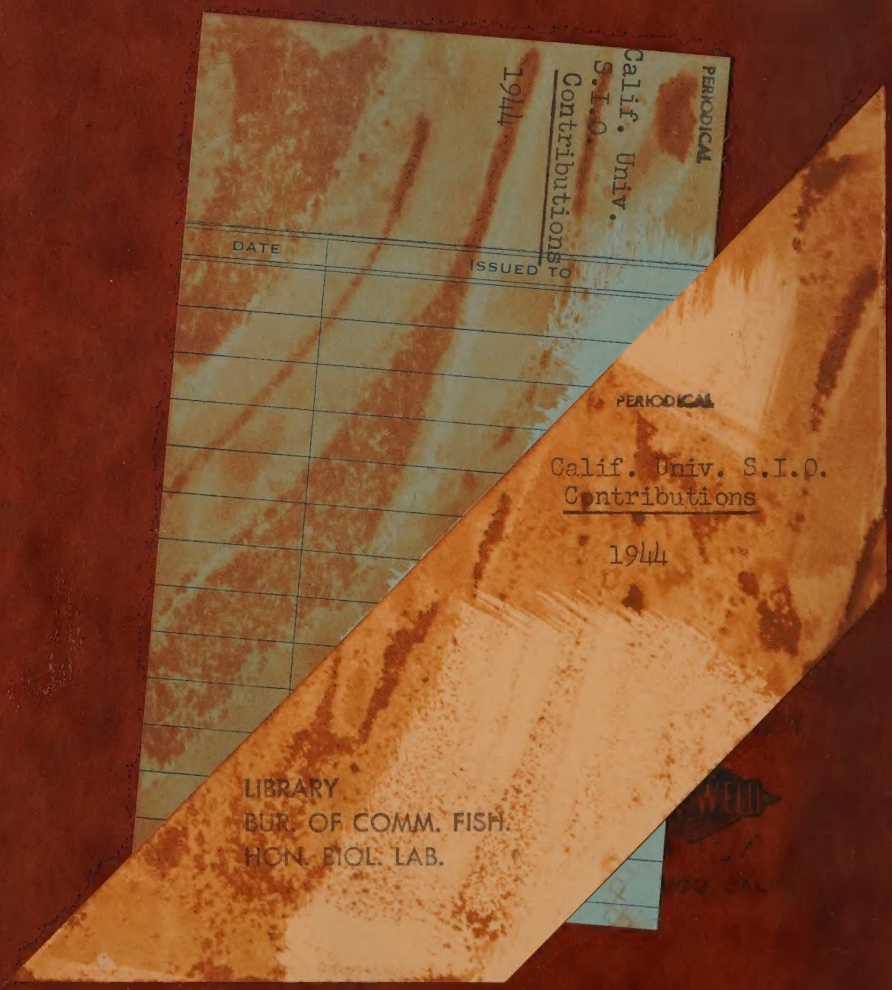
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LA JOLLA, CALIFORNIA

March, 1945

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Contribution No. 212

Reprinted from The Scientific Monthly, January, 1944, Vol. LVIII, pages 24–32.

AGAR: A VALUABLE SEAWEED PRODUCT

By C. K. TSENG

The importance of agar[1] in culture media for bacteriological and other microbiological research is well known in the scientific world. The United States, like other countries, used to depend principally on Japan for her agar supplies. Under the present conditions this source of agar is no longer available. The resulting agar shortage has become the concern of the Government. Consequently much attention has been given to problems regarding agar by various scientists—bacteriologists, botanists and chemists. There are already several articles recently published in *Science* and elsewhere, on methods for reclaiming used agar, on new sources of agar, and on the preparation of agar substitutes.

Agar: History and Nomenclature

In undertaking certain research problems with regard to the agar resources on the Pacific coast, the writer found a great deal of confusion existing in the use of the term agar. There is also misinformation about the sources of agar. The confusion is mostly due to the fact that the word "agar" or "agar-agar" was originally the Malay name for certain East Indian red algae, but is now almost exclusively used in science to designate the dried gelatinous extract derived from other seaweeds and produced principally in Japan. Thus, contemporary scientists writing on agar as we understand it nowadays not infrequently quote facts concerning *agar* as the word was used originally. Therefore, in order to avoid further confusion in this matter, it is necessary to standardize the application of the word "agar," restricting its use only to the dried extract. For the sake of simplicity the word "agarphyte" is here suggested to apply to the seaweeds used in its manufacture, instead of the more elaborate combinations of words such as "agar-bearing seaweeds," "agar-producing seaweeds," and "agariferous seaweeds."

The Original Agar. The original Malay word, *agar* or *agar-agar,* applies to certain common edible red algae, especially *Eucheuma muricatum* f. *depauperata* (= *Eucheuma spinosum*) of the Dutch East Indies, but is used indiscriminately for other species of the same genus. *Eucheuma muricatum* has been exported as *agar-agar* from the Dutch Indies to China for more than a century. It is quite common on Chinese markets, known under the popular name *Chilin-ts'ai,* meaning "Unicorn Vegetable." Quite erroneously, most authors have assigned the name "agar-agar" principally to *Gracilaria lichenoides,* which, to the knowledge of the writer, has never been used in China for making agar or in Ceylon where agar has never been made.

Introduction of Agar into Bacteriology. The introduction of agar into bacteriology has been discussed in detail by Hitchens and Leikinde. These authors credit the discovery of agar as a medium for bacteriological culture to a housewife, Frau Franny Eilshemius Hesse, who suggested to her husband, a bacteriologist, the use of agar-agar "which she had been using for years in her kitchen in the preparation of fruit and vegetable jellies." This discovery was communicated by Dr. Hesse to Robert Koch, "probably late in 1881." Because agar gel is relatively inert, is not liquefied by most bacteria, and remains in firm consistency at the incubation temperatures for the growth of various types of bacteria, it has helped greatly to advance the science of bacteriology.

It is, of course, impossible now to determine what was actually the "agar-agar" which Frau Hesse and eventually Koch used in this important discovery. Hitchens and Leikinde make this remark: "While yet in America she had received the recipe from her mother; her mother in turn had obtained the formula from some Dutch friends who

[1] "Agar" is preferred to "agar-agar" for the sake of simplicity. The word is pronounced in Malay with both *a*'s as in "arm." To follow the native Malayan tongue strictly the *r* should be pronounced like that in German. In this paper, whenever the word agar refers to the original Malay meaning, it is italicized.

DIVER DESCENDING WITH BASKET TO HARVEST AGARPHYTES*

had formerly lived in Java." From this note it may be reasonably inferred that the material used was the true East Indian *agar-agar*—the seaweed *Eucheuma muricatum,* rather than the dried extract from Japan.

The writer has as yet no definite information as to when the Japanese preparation was exported to Europe in quantities, and when it began to be called by the Malay term. From evidences in the writings of botanists and chemists in the 19th century, it seems that the Japanese product was already known by the European scientists in the 1860's, that it was then a very rare article—probably not something which the housewives could use in their kitchens—and that it still had no connection with the word agar, which was invariably used to designate certain East Indian algae, especially *Eucheuma spinosum.* As late as 1893 the Japanese government in the World's Columbian Exposition still called this *Gelidium* extract merely "dried seaweed jelly," instead of agar-agar as might be expected if it were already known by that name and exported as such in quantities to the Occidental countries.

Modern Use of the Word Agar. At the beginning of the present century the Japanese scientists, such as Yendo, had already called this peculiar Japanese product "agar-agar." Somehow, because of the persistence of the bacteriologists, the scientific world has generally accepted as agar, without further questioning into its origin, the dried gelatinous *Gelidium* extract in the form of powders, flakes, strips, or blocks from Japan, rather than the seaweed *Eucheuma* itself, the true *agar-agar.* Using the term as it is now generally accepted by scientific workers and dealers, we must give the seaweeds from which this peculiar extract is commercially prepared a separate name—"agarphyte" as proposed here—in order to avoid further confusion in the matter of terminology.

Definition of agar. It is to be noted that the definitions of agar that have appeared in various contemporary standard works are partly incorrect and as a rule too vague, because they apply not only to agar but also to other red seaweed extracts, such as carrageenin from Irish moss. Although our present knowledge of agar is still too incomplete to warrant a definition that would be acceptable to all scientists, it is advisable that

* All photographs courtesy U. S. Fish and Wildlife Service.

a revised definition be available. Such a definition should be broad enough to include products most scientists would agree to call agar, but precise enough to differentiate it from closely related but definitely different products, such as carrageenin mentioned above. Therefore, the writer ventures to offer the following definition for agar: The dried amorphous, gelatin-like, non-nitrogenous extract from *Gelidium* and other red algae, being the sulfuric acid ester of a linear galactan, insoluble in cold but soluble in hot water, a dilute neutral solution (1 to 2 percent) of which sets, upon cooling, to a firm gel solidifying at 35° to 50° C., and melting at 90° to 100° C.

Agar Production and Consumption

There has been a good deal of misinformation in the literature about the sources of agar. For instance, Tressler writes in 1923: "Most of the agar of commerce comes from Japan, China, Malaysia, Ceylon and neighboring coasts." At that time none of these countries except Japan, of course, produced any commercial agar. One botanist makes the following remark in a publication in 1917: "In the past agar-agar has been made principally in Germany from algae obtained from Japan." To the writer's knowledge Germany has never produced any agar so far, although she has made a purified agar from the Japanese product. Such misleading notes have been quoted again and again in current literature.

Japan is most probably the first country to produce agar in its present form. There are several theories as to when the present method of agar manufacture involving freezing and thawing was discovered, and according to Horiuchi, the following four years have been suggested: 1647, 1655, 1658 and 1688. Recently Chase reported, "The Chinese introduced it to Japan in A.D. 1662." In a recent communication she wrote the writer that her information was based on an old Japanese encyclopedia. The confirmation of any of these theories will be of some historical interest.

Japan is, and probably will always be, the leading agar producing country, in view of the vast quantity of agarphytes produced on her extensive coast, her well established agar industry, her low cost of production, and the appreciation of agar as food by her people. Agar is produced principally in the Osaka, Hyogo, Kyoto, Nagano, Yamanashi and Gifu prefectures where the climate is favorable for its manufacture by the old method. Some agar is also produced in Karafuto (Saghalien) where it is extracted from a different material by a different method. According to Horiuchi in 1936, there were 512 establishments with a total output of 679,918 kans or 5,622,921 lbs., worth 9,712,-497 yen, or about $2,400,000. The total export that year was 3,921,638 lbs., worth 5,574,452 yen, or about $1,400,000.

Up to the outbreak of war in 1937, China had a small agar industry consisting of three factories located at Ningpo, Tsingtao and Chefoo. No published data were available as to their total annual output. On the basis of information obtained from two of the firms and of the amount of agarphytes produced in China, the writer estimates the Chinese annual production at about 75,000 lbs., which normally was worth about 200,000 yuan, or approximately $50,000.

The United States has been making agar for about 28 years. The annual production has been very irregular, varying from 1,802 lbs. (1934) to 44,895 lbs. (1930), and in one exceptional year (1925) was as much as 117,773 lbs. Until 1941, when the domestic production was about 52,000 lbs., only one factory was actively engaged in making agar. The war has greatly stimulated the growth of this industry and now there are five concerns devoted to agar production, all located in southern California. The expected annual output will total 200,000 to 250,000 lbs.

In recent years Russia has also paid much attention to the production of agar from local seaweeds. According to Kizevetter, Vladivostok and Archangel each produced 44,000 lbs. and Vladimir 33,000 lbs., making a total output of 121,000 lbs in 1936. Besides, Odessa in the Black Sea was reported to have produced 440,000 lbs. of "agaroid" in the same period. This substance is probably of the nature of a seaweed gum similar to carrageenin. If, however, it proves to be a true agar as defined above, the Russian production in 1936 would have far exceeded

that of the United States even at the present rate of production.

The agar industry in the Netherlands Indies is a recent one. There were reported to be two factories in 1938. The total output was not known. At present both Australia and New Zealand are very actively investigating their agar resources. Recently Wood reports that an Australian firm is producing its own requirements for meat canning and that others will be making "agar" soon. This "agar" is undoubtedly in the form of a gel which is directly used in canning without passing through the freezing-thawing-drying processes, as the same author remarks, "no dried agar has yet been produced." Information reaching the writer reveals that South Africa has a rich natural resource of *Gelidium* and other agarphytes, still untapped, and enough to develop a great agar industry. A firm at Capetown was reported to have produced some agar-gel which was used directly in making confectionery. Perhaps these countries are producing some true agar by now. In Baja California, Mexico, from which came the majority of the *Gelidium* used in the United States in 1942, a small factory with a capacity of a ton of dry *Gelidium* per month is already in operation, and a larger one is being built.

On the basis of these data and information the writer estimates the world production of agar at six million pounds per annum of which 90 to 95 percent is made in Japan.

Japan also leads the world in the consumption of agar. According to Horiuchi, out of the total production of 2,207,378 lbs. in 1902, 366,542 lbs., or 16.6 percent, were consumed in that country. In 1936, while the production increased 2.6 times to 5,622,921 lbs., the consumption increased even more significantly by 5.7 times to 2,112,736 lbs., constituting 37.6 percent of the total production and about 0.35 ounces per capita. Agar consumption in China used to be over a million pounds per annum up to the early part of this century. Of the total Japanese export of 1,767,528 lbs. in 1906, 1,284,633 lbs., or about 73 percent, went to China and Hong Kong. In recent years, because of the enormous increase of price of agar, because of some local production, and because of the strained relation between China and Japan,

HAULING IN A BASKETFUL OF *GELIDIUM* WEIGHING ABOUT 65 POUNDS

the Japanese agar export to China has declined drastically. In 1938 China, including Hong Kong, imported only 109,592 lbs. Normally China would consume at least six to eight hundred thousand pounds annually for food alone.

The United States imports of Japanese agar during the last few years before the outbreak of war fluctuated between 500,000 and 700,000 lbs. In 1941, besides making 52,000 lbs., she imported 565,059 lbs. from Japan and 32,413 lbs. from China. According to Kizevetter, Russian consumption is about 660,000 lbs. per annum. In 1938 Germany imported 596,239 lbs., France 442,225 lbs., England 294,329 lbs., Dutch East Indies 162,393 lbs., Australia 144,571 lbs. and Strait Settlements 58,520 lbs., all from Japan. These imports represented, presumably, also the amounts consumed by these countries. It is to be noted, however, that the Dutch East Indies and the Strait Settlements have long been using a large quantity of local seaweeds to prepare their own agar gel.

THE AUTHOR GOES UNDER

DIVING TO STUDY GROWTH RATE AND QUANTITATIVE PRODUCTION OF *Gelidium* ON CALIFORNIA COAST.

Uses of Agar in Medicine and Science

Agar originally was used in making jelly and certain food preparations in the Orient. Since 1882, when it was first introduced into bacteriology, its technical uses have increased enormously.

As Microbiological Culture Medium. This is the most important use of agar, so far as human welfare is concerned, although the quantity of agar actually employed in this way constitutes only a minor portion of the total. Undoubtedly agar has received more respect and honor in this service than in all the others put together. Its chief value as a culture medium lies in the firm consistency of its gel at incubation temperatures for the growth of bacteria and other micro-organisms, and in its inertness towards most bacteria. These were the two main reasons which motivated bacteriologists in the latter part of the last century to prefer agar to gelatin as a culture medium. Agar is also remarkable for melting at about 95° C. and solidifying at about 40° C. Because it remains liquid when cooled to 42° C., organisms may be thoroughly mixed with it at a temperature that does not injure them; on the other hand, once it gelifies, it will remain firm at about the same temperature, usually 37° C., at which the pathogenic forms are generally incubated. The reversibility of the agar gel enables the operator alternately to warm it into the sol state and cool it into the gel state. Its firm, rubbery surface is not easily ruptured when organisms are streaked across it by needle. Pure agar gel is nearly transparent and neutral in reaction. All these unique characteristics of agar make it unexcelled as a bacteriological medium. It is also widely used in the preparation of semi-solid media. Having a ten times greater jellifying power than gelatin, it is a much cheaper material to use. Nowadays, not only bacteriologists, but also other microbiologists, as well as scientists working on pure culture and life history of fungi, algae and even orchids, have to depend greatly on agar media.

As Laxative. The popular use of agar as a laxative is based on its ability to absorb and hold water, becoming at the same time a lubricant and a mild stimulant. For this purpose it may be taken in various ways—

PRINCIPAL AMERICAN AGARPHYTE
A FEMALE PLANT OF *Gelidium cartilagineum.*

"HAIR AGAR"
AN ASEXUAL PLANT OF *Gelidium nudifrons.*

with cream and sugar, or in special chocolate-coated preparations, or as a mineral-oil emulsion. *Merck Index* remarks that "unlike drug cathartics, it is not habit-forming, and does not lose its efficiency or require increasingly larger doses." According to Chase, quoting Caravaggi and Manfredi, agar also contains certain laxative and purgative principles which act uniquely on the intestine by exciting peristalsis without affecting the functions of the stomach or the duodenum.

In Dental Impression Mold. In dental work agar has proved to be the best material for making impression mold. Although materials made of algin are recently being used to take the place of agar, dentists mostly agree that, if the latter were available, they would go back to it. The value of agar gel lies in its having a long temperature lag between liquefying and hardening, which factor is very important in this kind of work. For this purpose generally a very concentrated agar gel is used to which a dye and a disinfectant are added. In using it a small portion of the preparation is heated to become a fluid, cooled down to about room temperature, placed in the tooth socket or in the oral cavity, cooled thoroughly, and upon solidification the wanted impression is obtained.

Other Medical Uses. Agar has long been used to stabilize an emulsion to carry vitamins and other items like acidophilus milk. It has also served in wound dressing on account of its inhibitory action on blood-clotting. Used in a preparation applied beneath the bandage, it helps to quicken the healing process. Glycerine suppositories have been made with agar as a vehicle. In obesity it is said to be useful. According to *Merck Index* it is also used in glycerine jelly for chapped hands and in prosthetic work in surgery.

Agar as Food

As Food in the Orient. Agar has been and is used as a food in a great variety of ways in China and Japan. It should be noted, however, that agar has little nutritive value. A "food" in an Oriental sense may not necessarily be nourishing. Only the principal foods, like rice, need have nutritive value. The complementary foods, such as agar, may be merely appetizing. Up to the

early part of this century China had been importing from Japan annually over a million pounds of this commodity entirely for food. It is frequently served as a substitute for the expensive birds' nests. For this purpose the best grade of agar, which will not dissolve too readily in the hot soups, has to be used. It is also used in a special kind of "cold dish"; the agar strips are cut into smaller pieces, washed by soaking in cold water, placed in hot water, taken out before they show any signs of going into solution, and then mixed up with some good grade of soya sauce, some black vinegar and sometimes a few other ingredients such as red pepper. In Japan, agar is also cooked with rice, and with seasoning it forms an "agar-rice." Sweetened and flavored agar-gel is a favorite "cool diet" in the summer in hot countries such as South China and Malaya.

As a Food Adjunct. In recent years agar has been used extensively as an adjunct in various food preparations. Generally speaking it serves as a substitute for gelatin where the latter, for one reason or another, is unsuitable; it has also proved to be very economical, since, on account of its high gel strength, a relatively small amount is needed. It is used in confectionery throughout the world wherever agar is known. The confectioner can make a healthful jelly candy with less sugar by using agar in about half to one percent solution. In the preparation of drinks, such as malted milk, it provides a healthful body and acts as a stabilizer. Added to bread and pastries it helps to keep them from drying out, on account of its moisture retaining ability.

In the manufacture of frozen dairy products, agar has been most successfully used in sherbets and water-ices. It makes them smooth and prevents the drainage of unfrozen sirup out of them in the hardening room. Unlike gelatin, it acts quickly, and aging of products containing agar as the stabilizer is unnecessary. It making ice cream it also imparts a smoother texture to the preparation. The use of agar has helped to make a firmer bodied cheese of improved slicing qualities. Desserts and salad jellies made with agar have the distinctive advantage of setting rapidly and certainly, even in very warm climates. Being unassimilable and having high water absorbing capacity,

AGARPHYTES GROWING ON ROCKS NEAR LA JOLLA COVE, CALIFORNIA
Gelidium cartilagineum FLOURISHES IN EXPOSED PLACES WHERE WATER IS TURBULENT AND FAST.

it is an excellent roughage, recently used extensively in many breakfast and "health" foods.

In Meat and Fish Canning. In terms of agar consumption per capita, Australia is second only to Japan, using about fifteen hundred thousand pounds, or about 0.25 ounces per person per annum. The major part of this agar is said to be employed in meat and fish canning. It helps to keep the softer types of meat and fish from falling apart. Its ability to retain its gelatinizing power in a greater extent than animal gelatin, after subjection to the high temperature necessary in processing these foods, makes it superb for this particular purpose. New Zealand normally uses thirteen tons per annum for canning sheep's tongues. It is the writer's conviction that the use of agar in meat and fish canning will become more and more popular in the years to come. At present it is not being utilized in this way in this country as much as it should be.

In Meat and Fish Preservation. Agar provides a rather simple but effective way of temporarily preserving easily spoiled foodstuffs like meat and fish. It is especially recommended for hot countries where refrigeration is not available. The meat to be preserved is cooked with some agar and the resulting meat-gel will remain wholesome for quite a long time. The agar is not attacked by most putrefying bacteria, and thus it helps to prevent the entrance of oxygen and bacteria which will otherwise spoil the meat.

Agar in Industries

In early days agar was extensively used in oriental industry in fabric sizing and in rendering rice paper more durable. It was also employed in making waterproof paper and cloth, which were used in China to wrap expensive porcelains and bronzes for exporting to Europe. In other countries such waterproof wrappings also served to prevent corrosion of munitions.

At present agar is still being used in the textile industry, but only in silk sizing and in the warp of silk. In the modern paper industry agar is utilized to some extent as a paper coating to impart resistance to the penetration of resin, wax and grease. It is used to make the gelatinous rolls in hectograph duplicators. In the tanning industry, it serves to impart a gloss and stiffness to leather in the finishing process. It is employed as an ingredient of calcimine and shoe stains. Occasionally it is also used in the making of molds required by workers in plaster of Paris.

Agar also renders its services to agriculture as a supplement in nicotine sprays for insect pest control. In the cosmetic industry agar is used as the base of certain non-grease preparations, shaving soaps and hand lotions. It is also employed as a binder in certain dentifrices. The least expected, but important, use of agar is in the electrical lamp industry. In the hot drawing of tungsten wire, a lubricant is necessary to facilitate the drawing operations and reduce the wear on the die. Westinghouse uses a lubricant made of homogeneous suspension of powdered graphite in agar gel. In Japan, agar is also employed in making electric lamp shades.

Among the recently discovered promising uses of agar is its application in making photographic plates and films. Actually, more than twenty years ago patents were already issued in Germany covering such use of agar. That agar was not extensively used for this purpose was due to the fact that certain chemicals present in gelatin, but not occurring in agar, were necessary in making the sensitive emulsion. Recently it was found that these substances could be successfully added to agar. With this problem solved, agar is said to be superior to gelatin for photographic films because an agar film need be only one eighth the thickness of a gelatin film, is less soluble in water, does not melt in the tropical heat, and is cheaper. One firm is said to assert that agar is a better substance for supporting sensitized emulsion than any similar material now on the market.

There are undoubtedly many more uses of agar of which the writer is not aware and the number of its uses is expected to increase. At present no published data are available as to the amounts of agar used annually for the above mentioned purposes. According to information from agar dealers and various other sources, the 650,000 lbs. of agar used annually in the United States before the war were distributed approximately as follows:

Laxative	100,000
Microbiological culture medium	100,000
Bakery	100,000
Confectionery	100,000
Dental impression mold	75,000
Meat packing	50,000
Emulsifier	50,000
Cosmetic	25,000
Miscellaneous	50,000

Kinds and Sources of Agarphytes

There is a good deal of misinformation regarding the different kinds of commercial agarphytes and their geographical sources. *Gracilaria lichenoides* (=*Sphaerococcus lichenoides, Plocaria candida*), which has undoubtedly been mentioned the most frequently by writers reporting on agarphytes, is actually a very unimportant and negligible source for commercial agar. Another commonly cited plant for making agar is *Gloiopeltis tenax* (=*Sphaerococcus tenax, Plocaria tenax, Gigartina tenax, Gracilaria tenax*). It has never been used for this purpose. The genus *Gigartina* has been credited with a share in agar manufacture; again, not a single species has ever been commercially utilized in this manner, so far. The "Gelidium corneum," supposed to be the principal agarphyte, is still a taxonomic mystery remaining to be solved; it should be removed from the list of agarphytes until its true identity is revealed. According to Kizevetter and others, *Phyllophora rubens* is used in making "agaroid," which may not be a true agar, and is therefore excluded at present.

The list of agarphytes is certainly a lengthy one. There are, however, only a few which can be said to be the principal sources of agar: *Gelidium Amansii, G. cartilagineum,* and *Ahnfeltia plicata.* Besides these there are about forty other red algae, most of which are used merely as supplementary materials to mix with the principal agarphyte in agar manufacture. The American factories use almost exclusively *Gelidium cartilagineum* (L.) Gaill. which forms the "agarweed" of the commercial divers.

AGAR-PRODUCING SEAWEED
Gracilaria confervoides FROM SAN DIEGO BAY.

Contribution No. 216

Reprinted from JOURNAL OF THE WASHINGTON ACADEMY OF SCIENCES
Vol. 34, Nos. 1 and 2, January 15 and February 15, 1944

ZOOLOGY.—*Geographical distribution of the nemerteans of the Pacific coast of North America, with descriptions of two new species.*[1]

WESLEY R. COE, Scripps Institution of Oceanography. (Communicated by WALDO L. SCHMITT.)

An examination of the nemerteans in the collections of the United States National Museum revealed two species from the Pacific coast of North America believed to be new to science, in addition to many others from new localities. Most of them were dredged on the cruises of the U.S. Bureau of Fisheries steamer *Albatross* off the coasts of California, Washington, and Alaska and in the seas adjacent to the Japanese islands. These records are included in the following list, which shows the habitat and geographical distribution of each of the Pacific coast species so far as known at the present time. This list will supplement the data contained in the "Revision of the nemertean fauna of the Pacific coasts of North, Central and northern South America" (Coe, 1940). The total number of species now known from the Pacific coast of North America is increased to 99. Of these, 65 have been found only on the Pacific coast of North America, including Bering Sea and the adjacent Arctic coast of Alaska; 16 occur also on the coasts of Japan; 11 on the Atlantic coast of North America; 14 in European waters; 5 on South African shores; and 7 on the Pacific coast of South America.

Order PALEONEMERTEA
Family Tubulanidae

1. *Tubulanus albocinctus* Coe. Among red algae at depths of 100 to 200 meters; off coast of southern California.

2. *T. capistratus* Coe. Intertidal zone; coast of Alaska to Monterey Bay, Calif.; Japan. One specimen nearly a meter in length was collected by the *Albatross* in 1906 near Hakodate, Japan.

3. *T. cingulatus* Coe. Yes Bay, Behm Canal, Alaska, 290–400 meters; intertidal zone; Monterey Bay, Calif.

4. *T. frenatus* Coe. Intertidal zone; southern California.

5. *T. nothus* Bürger. Intertidal zone to 40 meters; coast of Alaska; South Africa; Mediterranean. The Alaska records are from preserved specimens only, and there remains the possibility they may actually have represented *T. annulatus* (Montagu), which is similar in markings and which is widely distributed on northern coasts from Greenland to Norway, Great Britain and the Mediterranean and has also been reported from South Africa.

6. *T. pellucidus* Coe. Intertidal zone; coasts of New England and southward; Monterey Bay to San Diego, Calif.

7. *T. polymorphus* (Renier). Intertidal zone; northern coasts of Europe; Mediterranean; Aleutian Islands, Alaska, British Columbia to Monterey Bay, Calif.

8. *T. sexlineatus* Griffin. Intertidal zone; Alaska to southern California.

9. *Carinomella lactea* Coe. Intertidal zone to 20 meters; Monterey Bay to San Diego, Calif. Burrowing form, found on sandy shores of boys.

Family Carinomidae

10. *Carinoma mutabilis* Griffin. Intertidal

[1] Contributions of the Scripps Institution of Oceanography, University of California, new ser., no. 216. Received October 27, 1943.

zone to 40 meters; British Columbia to Gulf of California. Burrows on sandy shores of bays.

Family Cephalothricidae

11. *Cephalothrix major* Coe. Intertidal zone; southern California.

12. *Procephalothrix spiralis* Coe. Intertidal zone to 20 meters; New England; Alaska to San Diego, Calif.

Order HETERONEMERTEA
Family Baseodiscidae

13. *Baseodiscus delineatus* (Delle Chiaje). Widely distributed in both Northern and Southern Hemispheres; Mediterranean to Cape Verde Islands; Mauritius; Bermuda; Barbados; Fiji Islands; Chile; Japan; Gulf of California.

14. *B. delineatus* var. *curtus* (Hubr.). Coextensive with the preceding.

15. *B. mexicanus* Bürger. Gulf of California; west coasts of Mexico and Panama; Galápagos Islands; intertidal zone to 100 meters; among shells and corals.

16. *B. princeps* Coe. Alaska to Puget Sound; intertidal zone and off shore to depths of 50 meters or more; off Goloi Island, Alaska, 50 meters; off Oshima, Japan, 250 meters (*Albatross*); Sea of Japan 135–290 meters (*Albatross*); off Ose Saki, Japan, 260 meters (*Albatross*); south of Hokkaido, Japan, 340 meters (*Albatross*). This species was also found by Yamaoka (1940) in the intertidal zone at Hokkaido, Japan, but was erroneously assigned by him to *B. curtus* Hubr. Supplementing the original description by Coe (1901) it has since been ascertained that some individuals of *B. princeps* on the coast of Alaska and in Puget Sound have the lateral margins and ventral surface of the head white or colorless. These white areas disappear when the head is strongly contracted.

17. *B. punnetti* Coe. Monterey Bay to Gulf of California, Mexico; intertidal zone to 380 meters.

18. *Zygeupolia rubens* (Coe). Intertidal zone to 50 meters; southern New England and southward to North Carolina; Monterey Bay, Calif., to Ensenada, Mexico.

Family Lineidae

19. *Euborlasia maxima* Coe. Gulf of California.

20. *E. hancocki* Coe. Coasts of Mexico, Panama, and Peru; 17 to 100 meters.

21. *E. nigrocincta* Coe. San Francisco Bay, 20 meters; Monterey Bay, Calif., to Ensenada, Mexico; intertidal zone to 30 meters.

22. **Euborlasia variegata**, n. sp. This species was represented in a collection of nemerteans from Alaska by a headless fragment about 160 mm in length. The width of the body is 7 to 10 mm and the thickness 6 to 8 mm. The body is somewhat flattened in the middle intestinal region but becomes rounded posteriorly. The posterior extremity is rounded and without caudal cirrus.

This specimen is put on record because of the remarkable and distinctive coloration of the body. In spite of preservation in alcohol for about two years the color pattern is still conspicuous, consisting of an orange ground color, overlaid with bluish black longitudinal bands and transverse rings. On some portions of the body the black pigment covers most of the dorsal and ventral surfaces but in other portions it is confined to narrow rings which encircle the body. Several adjacent rings become wide and confluent on dorsal or ventral surface, or both, giving the appearance of broad, interrupted longitudinal bands connected by narrow lateral rings. This specimen represents a ripe female and the abundance of ova presumably influences the color pattern to some extent.

Sections show that the bluish-black pigment is confined to the cutis and that the pattern is formed by the relative thickness of the pigment layer, which is thin in certain areas and much thicker and denser in others. The epithelium and the muscular layers are colorless or yellow and the intestinal epithelium and ova deeper yellow or orange.

This specimen (U.S.N.M. 20633) was dredged at a depth of about 30 meters in Port Graham, Cook Inlet, Alaska, by Dr. Waldo L. Schmitt in connection with the Alaska King Crab Investigation, 1941.

23. *Lineus bilineatus* (Renier). Northern coasts of Europe; Mediterranean; Madeira; South Africa; Alaska to San Diego, Calif.

24. *L. flavescens* Coe. Southern California to Gulf of California, Mexico.

25. *L. geniculatus* (Delle Chiaje) (=*L. digueti* Joubin). Intertidal zone to 30 meters; Gulf of California; west coasts of Mexico and Panama; Mediterranean and Black Seas; west coast of Africa.

26. *L. pictifrons* Coe. Intertidal zone; Puget

Sound to coast of Mexico.

27. *L. ruber* (O. F. Müller). Intertidal zone to 10 meters; circumpolar; Siberia; northern coasts of Europe; Mediterranean: Madeira to South Africa; Greenland to southern New England; Alaska to Monterey Bay, Calif.

28. *L. rubescens* Coe. Monterey Bay to San Diego, Calif.

29. *L. torquatus* Coe. Coast of Alaska to San Francisco Bay.

30. *L. vegetus* Coe. Found in the intertidal zone beneath stones and decaying vegetation in estuaries, harbors and bays, as well as in crevices of rocks and among corallines and other growths exposed to the full force of the surf; sometimes above middle of intertidal zone; occasionally in brackish water. Commonly associated with dead barnacles and mollusks; feeds on ciliates and other small organisms, living or dead. San Francisco Bay, Calif., to Ensenada, Mexico. Reproduces asexually by fragmentation as well as sexually by fertilized eggs; has remarkable regenerative capacity.

31. *Micrura alaskensis* Coe. Intertidal zone; Prince William Sound, Alaska, to Ensenada, Mexico; Japan.

32. *M. impressa* (Stimpson). Bering Strait.

33. *M. nebulosa* Coe. Dredged at depths of 120–900 meters off the coasts of Alaska and California.

34. *M. nigrirostris* Coe. Among kelp holdfasts and other growths on rocks at low-water mark and below; southern California.

35. *M. olivaris* Coe. Monterey Bay and off San Francisco, Calif.; low-water mark to 120 meters.

36. *M. pardalis* Coe. Intertidal zone; Monterey Bay, California, to Ensenada, Mexico.

37. *M. verrilli* Coe. Intertidal zone and below; Alaska to Monterey Bay, Calif.

38. *M. wilsoni* Coe. Intertidal zone to 35 meters; Monterey Bay, California, to Gulf of California.

39. *Cerebratulus albifrons* Coe. Muddy flats between tide marks and below to depths of 100 meters or more; Alaska to San Diego, Calif.

40. *C. californiensis* Coe. On muddy shores and in bays to depths of 35 meters or more; Puget Sound to Gulf of California.

41. *C. herculeus* Coe. Bering Sea, coast of Alaska to central California and off the coast to depths of 60 meters or more.

42. *C. lineolatus* Coe. Muddy bays, southern California, Gulf of California and west coast of Mexico; intertidal zone to 70 meters or more.

43. *C. longiceps* Coe. Intertidal zone; Yakutat Bay, Alaska; off Oshima, Japan, 250 meters.

44. *C. marginatus* Renier. Sandy and muddy shores to depths of 100 meters; circumpolar; Norway to Madeira; Greenland and Labrador to Cape Cod; Alaska to San Diego, Calif.; Bering Sea (62°N. 173°W.), 70 meters; Japan.

45. *C. montgomeryi* Coe. Coast of Siberia; Bering Sea; Alaska to Monterey Bay, Calif.; Behm Canal, Alaska, 150–400 meters; Moss Cape, Belkofski Peninsula, 40 meters; off Hokkaido, Japan, 600 meters.

46. *C. occidentalis* Coe. Alaska to San Francisco Bay; off central California, 120 meters; Cold Bay, Alaska, 40 meters; Bellingham Bay, Wash., 20 meters; intertidal zone, Prince William Sound to Puget Sound.

47. *C. signatus* Coe. Bering Sea, 110 meters.

48. *Diplopleura vivesi* Joubin. Gulf of California, Mexico.

Order HOPLONEMERTEA
MONOSTYLIFERA
Family Emplectonematidae

49. *Emplectonema bürgeri* Coe. Intertidal zone to 500 meters; Alaska to Monterey Bay, Calif.; off Vancouver Island, 300 meters; Chatham Strait, Alaska, 500 meters; off Oshima, Japan, 250 meters.

50. *E. gracile* (Johnston). Northern coasts of Europe to Madeira; Aleutian Islands and coast of Alaska to Ensenada, Mexico; Chile; Kamchatka to Japan; intertidal zone to 100 meters. In many localities on northern coasts the most abundant of all species of nemerteans.

51. *E. purpuratum* Coe. Aleutian Islands.

52. *Nemertopsis gracilis* Coe. Intertidal zone and below; Puget Sound to Ensenada, Mexico.

52a. *N. gracilis* var. *bullocki* Coe. Intertidal zone; coast of central California.

53. *Paranemertes californica* Coe. Monterey Bay, Calif., to Ensenada, Mexico; in sandy and muddy bays.

54. *P. carnea* Coe. Intertidal zone; Alaska to Puget Sound.

55. *P. pallida* Coe. Intertidal zone; Alaska.

56. *P. peregrina* Coe. Commander Islands; Aleutian Islands, Alaska, to Gulf of California; Kamchatka to Japan. Intertidal zone and below, among mussels and other growths; often very abundant.

57. *Dichonemertes hartmanae* Coe. Intertidal zone; San Diego, Calif.

Family Carcinonematidae

58. *Carcinonemertes epialti* Coe. Commensal parasite on crabs of the genera *Portunus*, *Pugettia* and *Euphylax*. Monterey Bay to San Diego, Calif.; Peru.

Family Ototyphlonemertidae

59. *Ototyphlonemertes spiralis* Coe. On sandy shores of bays; San Diego, Calif.

Family Prosorhochmidae

60. *Prosorhochmus albidus* (Coe). Intertidal zone; Monterey Bay, Calif., to Ensenada, Mexico.

61. *Oerstedia dorsalis* (Abildg.). Intertidal zone and below; circumpolar; Norway to Mediterranean; Madeira; Nova Scotia to Florida; Puget Sound to Gulf of California.

Family Amphiporidae

62. *Zygonemertes albida* Coe. Intertidal zone; British Columbia to Ensenada, Mexico.

63. *Z. thalassima* Coe. Intertidal zone; Alaska.

64. *Z. virescens* (Verrill). Intertidal zone and below to depths of 120 meters; Bay of Fundy, New England and southward to North Carolina; Puget Sound to Gulf of California.

65. *Amphiporus angulatus* (Fabricius). Circumpolar; Greenland; Davis Strait; Labrador to Cape Cod; Bering Strait; Bering Sea; Aleutian Islands and Kamchatka to Japan; Alaska; British Columbia; Puget Sound and southward to Point Conception, Calif.

66. *A. bimaculatus* Coe. Intertidal zone; Alaska to Ensenada, Mexico; off San Diego, Calif., 250 meters; Okhotsk Sea, 140 meters.

67. *A. californicus* Coe. Intertidal zone to 80 meters or more; coast of southern California.

68. *A. cruentatus* Verrill. Intertidal zone to 80 meters or more; New England to Florida; Puget Sound to San Diego, Calif.

69. *A. flavescens* Coe. Monterey Bay, Calif., to Ensenada, Mexico.

70. *A. formidabilis* Griffin. Bering Island, Aleutian Islands, coast of Alaska and southward to Monterey Bay, California.

71. *A. fulvus* Coe. Intertidal zone to 85 meters; southern California.

72. *A. gelatinosus* Coe. The absence of the proboscis in the type specimen did not permit a satisfactory description of this species (Coe, 1905). Specimens collected by the U.S. Fish Commission have since become available for study and in these the proboscis proves to be typical for the genus *Amphiporus*. The basis is pear-shaped, of moderate proportions and not quite so long as the rather slender central stylet which measures 0.16 to 0.18 mm in length in an individual 150 mm long. There are four pouches of accessory stylets, with 4–5 stylets in each, and 15 to 17 proboscidial nerves. The body contains a larger proportion of gelatinous tissue than has been reported for any other species of the genus. Length of body 100 to 150 mm; width 10 to 16 mm.

Dredged at a depth of about 300 meters southwest of Kodiak Island, Alaska; at 400 to 450 meters in Clarence Strait; at 40 meters near Port Townsend, Wash., and at 130 meters in Uraga Strait, Japan.

73. *A. imparispinosus* Griffin. Intertidal zone to 50 meters; coast of Siberia, Bering Sea, Alaska to San Diego, Calif., and Ensenada, Mexico. Abundant in many localities.

73a. *A. imparispinosus* var. *similis* Coe. Differs from the typical form in having 2, instead of 3, pouches of accessory stylets. Puget Sound to Ensenada, Mexico.

74. *A. macracanthus* Coe. Dredged in the Arctic Ocean off the northern coast of Alaska.

75. **Amphiporus maculosus,** n. sp. This species is distinguishable from others of the genus by the reddish brown spots and blotches on the dorsal surface. Another species, *A. nebulosus* Coe, has the dorsal surface more nearly covered with confluent dark brown spots and blotches, while in *A. maculosus* they are widely separated. *A. nebulosus* has 18 to 25 ocelli on each side of head but in the only specimen of *A. maculosus* available for study ocelli could not be detected. The stylet basis in *A. nebulosus* is much swollen posteriorly and about as long as the stylet, while in *A. maculosus* it is only moderately enlarged posteriorly and much longer than stylet. The nephridia, caecal diverticula and proboscis show minor anatomical differences.

Body moderately slender, narrowed posteriorly; head with inconspicuous oblique grooves. Length of type specimen 36 mm, width 3 mm, thickness 2 mm after preservation.

Color of body pale gray, with numerous reddish brown spots and blotches on dorsal surface; head without spots. These markings vary

in size from dots to large blotches, usually separated by much larger spaces without pigment. The spots in this specimen cover less than one-third the dorsal surface. Ventral surface pale gray.

Ocelli could not be detected either in the specimen cleared in oil or in the sections.

Proboscis sheath extends entire length of body. Proboscis large, stylet basis pear-shaped, about twice as wide posteriorly as anteriorly and twice as long as the posterior diameter. Central stylet two-thirds as long as basis. In this specimen there are 18 large proboscidial nerves. Each of the two accessory stylet pouches contains three stylets.

Cerebral sense organs large, situated anterior to brain, each with a relatively large canal leading anteriorly to an oblique groove on lateroventral surface near tip of head. Cephalic glands voluminous.

Nephridia extend anteriorly as far as posterior borders of cerebral ganglia. Intestinal caecum extends forward on ventral side of pylorus but terminates some distance posterior to brain; caecal diverticula short, not reaching brain. Gonads more numerous than intestinal diverticula; oviducts open ventrolaterally.

The single known specimen was collected at Lagoon Reef, St. Paul Island, Bering Sea. Type, U.S.N.M. no. 16797.

76. *A. nebulosus* Coe. Intertidal zone; coasts of Alaska and Japan.

77. *A. occidentalis* Coe. Dredged at depths of 70 to 170 meters off the coast of Washington.

78. *A. pacificus* Coe. Dredged at depths of 70 to 180 meters in the Bering Sea and off the coasts of Washington and California. In two specimens from Bering Sea the ocelli are more numerous than figured by Coe (1895) and are arranged in two groups on each side of head. The anterior, marginal, group on each side consists of about 10 large and 8 smaller ocelli, while the posterior, cerebral, group has about 8 large and 6 small ocelli. Most of the cups of those in the marginal groups are directed forward and those of the cerebral groups backward.

79. *A. paulinus* Punnett. Pribilof Islands, Bering Sea.

80. *A. punctatulus* Coe. Intertidal zone; Catalina Island, Calif.

81. *A. rubellus* Coe. Intertidal zone to 200 meters; coast of southern California.

82. *A. tigrinus* Coe. Intertidal zone; British Columbia and Puget Sound.

Family Tetrastemmatidae

83. *Amphinemertes caeca* Coe. Dredged with tunicates at a depth of 5 meters; Kodiak Island, Alaska.

84. *Tetrastemma aberrans* Coe. Intertidal zone; coast of Alaska.

85. *T. bicolor* Coe. Shallow water; Kodiak Island, Alaska.

86. *T. bilineatum* Coe. Intertidal zone; San Diego, California.

87. *T. candidum* (Müller). Circumpolar; Greenland and Norway to Madeira; South Africa; Labrador to New England and southward; Alaska to Ensenada, Mexico.

88. *T. nigrifrons* Coe. Intertidal zone; Puget Sound to coasts of Mexico and Costa Rica; Japan.

89. *T. quadrilineatum* Coe. Intertidal zone; Monterey Bay, Calif. to Ensenada, Mexico.

90. *T. reticulatum* Coe. Southern California; intertidal zone.

91. *T. sexlineatum* Coe. Dredged at a depth of 35 meters near San Clemente Island, Calif.

92. *T. signifer* Coe. Intertidal zone to 10 meters; Monterey Bay to San Diego, Calif.; locally common on kelp holdfasts.

POLYSTYLIFERA REPTANTIA

Family Drepanophoridae

93. *Drepanophorus crassus* (Quatrefages). Dredged at depths of 2 to 100 meters or more; Arctic Ocean; European coasts; tropical Pacific islands; Cape San Lucas, Mexico; Panama; West Indies; Peru.

94. *D. ritteri* Coe. Dredged at depths of 50 to 300 meters off coast of southern California.

POLYSTYLIFERA PELAGICA

Family Planktonemertidae

95. *Planktonemertes agassizii* Woodworth. Bathypelagic at depths of 1000 meters or more off coasts of Panama and Ecuador.

Family Nectonemertidae

96. *Nectonemertes pelagica* Cravens and Heath. Bathypelagic at depths of 100 meters or more off coasts of California and northern South America.

Family Pelagonemertidae

97. *Pelagonemertes brinkmanni* Coe. Bathypelagic at depths of 600 meters or more. Bering Sea and off coasts of Alaska, Aleutian Islands and Kamchatka.

Family Dinonemertidae

98. *Dinonemertes mollis* Coe. Bathypelagic at depths of 600 meters or more; off coast of Mexico.

Order Bdellonemertea
Family Malacobdellidae

99. *Malacobdella grossa* (Müller). Commensal in various species of bivalve mollusks. Northern coasts of Europe, Mediterranean; Nova Scotia to Chesapeake Bay; Puget Sound to California.

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Contribution No. 217

ZOOLOGY.—*Nemerteans from the northwest coast of Greenland and other Arctic seas.*[1] WESLEY R. COE, Scripps Institution of Oceanography. (Communicated by WALDO L. SCHMITT.)

A small collection containing 12 specimens of nemerteans was obtained in July, 1940, by Capt. Robert A. Bartlett at depths of 23 to 115 meters off the northwest coast of Greenland. The four species represented are of interest because none of them had been reported previously from that locality. All, however, had been collected formerly from other portions of the coast of Greenland and elsewhere in the Arctic. In this paper the distribution of each of these species as known at the present time is indicated, and a supplementary account is given of such organ systems as had heretofore been inadequately described. A list of the 30 other species that have been found in the Arctic is appended, with the geographical distribution of each.

Tubulanus annulatus (Montagu)

Gordius annulatus Montagu, 1804.
Carinella annulata Bürger, 1895, 1903.

One incomplete individual was dredged at a depth of 50 to 115 meters 1 mile northwest of Conical Rock. This specimen is 3 to 4 mm in width, indicating an individual having a total length of 20 to 30 cm when living.

This species is widely distributed on the eastern shores of the North Atlantic, from Norway and Great Britain to the Mediterranean; it has also been found in the South Atlantic, near the Cape of Good Hope (Stimpson, 1856). It is closely similar to *T. nothus* Bürger, which has likewise been found near the Cape of Good Hope (Wheeler, 1934). In the Arctic it was previously dredged near King Karl Land; also off Cape Platen and in the Karajek Fiord, Greenland. Only a few other species of nemerteans are known to have such an extensive geographical distribution.

Micrura purpurea (Dalyell)

Gordius purpureus spinifer Dalyell, 1853.
Micrura purpurea Joh. Müller, 1858; Bürger, 1903.

Four large individuals evidently belonging to this species were dredged at depths of 45 to 115 meters 1 mile northwest of Conical Rock, northwest Greenland. The specimens after preservation measured 60 to 90 mm in length and 4 to 5 mm in width, indicating a length in life of 150 mm or more. As is the case with many other invertebrates, these worms frequently reach a larger size in the Arctic than in warmer regions. Individuals from the coast of Scotland average considerably larger than those of the same species in the Mediterranean and if the specimens in this collection are correctly identified, those of the Arctic regions become even larger. The same condition holds for *Tubulanus annulatus*.

This species is common on the European coasts from Scotland to the Mediterranean. It occurs from the intertidal zone to a depth of 200 meters or more. In the Arctic it was previously reported from Karajak Fiord, Greenland; also from Hinlopen Strait at a depth of 80 meters.

Cerebratulus barentsi Bürger, 1895

One incomplete specimen measuring 11 mm in width was dredged at a depth of 24 meters off the north shore of Wolsterholm Sound, northwest Greenland. The deep reddish brown pigmentation of the body was still retained after preservation for three years.

This species is known only from Arctic seas, having been reported from Kara Strait, from the sea north of Spitsbergen, Hinlopen Strait, Karajak Fiord, Greenland, off Amsterdam Island, and elsewhere at depths of 40 to 1000 meters.

Amphiporus groenlandicus Oersted, 1844

The collection contained six specimens of this common Arctic species. These measured 60 to 80 mm in length and 4 to 6 mm in width. They were dredged off the north shore of Wolsterholm Sound, northwest Greenland at a depth of about 20 meters.

These specimens were without ocelli and agreed in all essential respects with the published descriptions of this well-known species.

[1] Contribution of the Scripps Institution of Oceanography, University of California, new ser., no. 217. Received October 27, 1943.

Serial sections of one individual showed that the internal anatomy conforms with that of other individuals described by Bürger (1895, 1903) from other portions of the Arctic seas.

Since Bürger's account contained no description of the armature of the proboscis nor of the reproductive organs, such descriptions may be included here. The stylet basis is rather slender, conical or elongated pear-shaped and about twice as long as the basal diameter. In these specimens the bases measure from 0.08 to 0.10 mm in length and 0.035 to 0.05 mm in diameter at the base.

The central stylet is nearly equal to the basis in length. With one exception the proboscis was provided with 2 pouches, each containing 3 to 5 accessory stylets. In one of the six specimens one of the pouches was divided into two parts. The number of proboscidial nerves varies from 16 to 18.

The cerebral sense organs are large and situated immediately anterior to the brain, with posterior extensions on the ventral sides of the dorsal ganglia. Large nerves unite them with the dorsal ganglia and from each of them a slender canal extends forward to open ventrolaterally in an oblique groove near the tip of the head.

The nephridia extend forward as far as the lateral borders of the brain. Near the posterior end of the nephridial system a large efferent duct opens ventrolaterally on each side of the body. The intestinal caecum extends forward nearly to the brain and sends lateral branches as far as the dorsal sides of the dorsal ganglia.

The gonads are much more numerous than the intestinal diverticula, as many as four or even six ovaries or spermaries being cut in a single transverse section of the body. They are situated both dorsally and ventrally to the lateral nerve cords, but the genital ducts with few exceptions open dorsolaterally.

Each of the six specimens was infested by protozoan parasites. These were most abundant within the blood vessels but others were imbedded in the adjacent connective tissue parenchyma.

This species is widely distributed in Arctic seas, having been reported from both the eastern and western coasts of Greenland, from Hinlopen Strait, Barents Sea, and from the waters off King Karl Land, Jena Island, Franz Joseph Land, and Spitsbergen at depths of 4 to 450 meters. A similar species, *A. caecus* Verrill, was dredged at a depth of about 35 meters off the New England coast north of Block Island, Mass. Coe (1943) suggested the possibility that the two supposed species may later prove to be specifically identical.

Other species previously reported from the Arctic seas include the following:

Tubulanus groenlandicus (Bergendal). North Greenland.

Lineus koalensis Uschakow. Barents Sea.

Lineus maris-albi Uschakow. White Sea.

Lineus ruber (O. F. Müller). Circumpolar; coasts of Siberia; Greenland; Norway and Great Britain to Mediterranean; Madeira and South Africa, Labrador to southern New England; Alaska to California.

Lineus saint-hilairi Uschakow. White Sea.

Micrura impressa (Stimpson). Bering Strait.

Micrura lithothamnii Uschakow. Kola Fiord.

Cerebratulus brevis Uschakow. White Sea.

Cerebratulus fuscus (McIntosh). Off the coasts of Greenland and elsewhere in Arctic seas; Great Britain and Norway to Mediterranean.

Cerebratulus greenlandicus Punnett. Greenland and North Greenland.

Cerebratulus marginatus Renier (=*C. fuscus* Verrill). From off King Karl Land, Bremer Sound, Hinlopen Strait, Amsterdam Island, and East Spitsbergen. This species has a wide circumpolar distribution, being found on European coasts as far south as Madeira; on the eastern North American coast southward to Cape Cod and farther south in the offshore current; on the western North American coast southward to southern California and in the western Pacific as far south as Japan.

Cerebratulus melanops Coe and Kunkel. Gulf of St. Lawrence and northward.

Cerebratulus rigidus Isler. Novaya Zemlya.

Cerebratulus zachsi Uschakow. White Sea and Kara Strait.

Emplectonema derjugini Uschakow. Kola Fiord, Barents Sea.

Emplectonema neesi (Oersted). Coasts of Greenland, Iceland, Norway and Great Britain to Mediterranean.

Nemertopsis actinophila Bürger. Coasts of Bären Island; Ross Island; King Karl Land; Lomme Bay; Hinlopen Strait; from low-water mark to 240 meters.

Amphiporus angulatus (Fabricius). This com-

mon and widely distributed Arctic species appears to have been described also by Verrill as *A. stimpsoni*, *A. heterosorus*, *A. multisorus*, and *A. superbus;* also by Punnett as *A. thompsoni* and in part as *A. arcticus*. Greenland, Baffin Bay, Davis Strait, Labrador, Nova Scotia, and southward to Cape Cod on or near the coast and farther south beneath the offshore Arctic current. On the west coast of North America the species extends from the Arctic Ocean through Bering Sea, along the coast of Alaska and southward to Point Conception, California. On the Asiatic coast it occurs from Kamchatka to Japan.

Amphiporus hastatus McIntosh. Coasts of southern Greenland and northern Europe.

Amphiporus lactifloreus Johnston. Shores of Arctic and North Atlantic Oceans, extending southward to the Mediterranean Sea and on the American coast to Cape Cod; intertidal zone to 200 meters.

Amphiporus littoralis (Uschakow), *Gurjanovella littoralis* Uschakow. Barents Sea, White Sea.

Amphiporus macracanthus Coe. Arctic coast of Alaska.

Amphiporus murmanicum Uschakow. Kola Fiord.

Amphiporus pulcher (Johnston). Coasts of Spitsbergen, Norway, and Great Britain to Mediterranean; Greenland to Massachusetts Bay. Some of the specimens described by Punnett as *A. arcticus* evidently belonged to this species.

Tetrastemma albicollis Uschakow. Kola Fiord.

Tetrastemma arctica Uschakow. White Sea, Novaya Zemlya.

Tetrastemma candidum Müller. Circumpolar; Greenland to Madeira; South Africa; Alaska to Mexico.

Tetrastemma laminariae Uschakow. Kola Fiord; Novaya Zemlya.

Uniporus borealis (Punnett). Davis Strait.

Drepanophorus crassus Quatrefages. Widely distributed in Arctic, Antarctic and Tropics; dredged at a depth of 250 meters near Franz Joseph Land; coasts of Europe, Madeira, Mauritius, Kerguelen, Samoa, Tonga, Panama, West Indies.

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THE NEMERTEAN GORGONORHYNCHUS AND THE FLUCTUATIONS OF POPULATIONS[1]

IN a paper (Wheeler, 1942) discussing the significance of the recent discovery of nemerteans with a type of proboscidial apparatus heretofore undescribed, the naive suggestion is made that the branched type of proboscis peculiar to Gorgonorhynchus may have originated within the past forty years from a form with a simple tubular organ. The only grounds for this conclusion were that two species of the new form were found almost simultaneously on the coasts of Australia, India and Bermuda and that they had not been reported by earlier investigators who had made collections in the same regions.

It was considered remarkable that a species now plentiful on the shores of Bermuda was not found either by Verrill or by Coe, who had searched the same localities near the beginning of the present century. Considering also the circumstance that certain other species of nemerteans which were found at Bermuda forty years ago have not been seen recently, Wheeler infers that one of them may have been the "mutation mother-species of Gorgonorhynchus by development from the eggs." What a thrill it would give to students of evolution if such an inference could be substantiated. But since that seems to the writer unlikely of realization, a simpler explanation and one more in harmony with the behavior of littoral populations may be appropriate.

There is, of course, a possibility that the species has but recently arrived at Bermuda on the bottoms of ships, as suggested by Zimmerman (1943) and also considered by Wheeler, but such a distribution of a burrowing worm with the habits of Gorgono-

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rhynchus seems improbable. More seriously to be considered is the factor of chance. The combined intertidal shorelines of all the Bermuda islands extend for more than fifty miles, along which are innumerable stones that would form suitable protection for the nemertean. It is safe to say that relatively few of these stones have even yet been lifted to expose the treasures which may lie beneath them. Perhaps it was only by a mischance that neither Verrill nor Coe happened to look under just those stones which concealed a worm with such an aberrant structural modification that neither could have failed to notice if such a worm had been seen. The writer is unwilling to admit that human eyes are any sharper to-day than they were at the beginning of the century.

It is obvious that the chance of finding a representative of a species will increase rapidly as the number of individuals in the population becomes greater. And it would be unusual if any population remained constant in numbers. Every observer of long experience has witnessed many such fluctuations in abundance from year to year.

Populations wax and wane; periods of abundance are followed by others of relative scarcity or complete disappearance (Clements and Shelford, 1939; Coe and Allen, 1937). Such periodicities are as characteristic of the populations of littoral worms as of other organisms. They are so familiar to all biologists that it seems unnecessary to mention specific examples.

New species of animals and plants are being discovered in familiar localities every year; numerous individuals of the new type may appear suddenly and later disappear with equal rapidity. Dominant populations may be replaced by others, quickly or over a period of years. An increase in one locality may accompany the decrease or the complete disappearance of the same species in certain other regions.

Some of the described species have as yet been reported on but a single occasion. Their discovery may have been due to the circumstance that one or more individuals wandered from the usual habitat of the species. As the population increases a wider dispersal is likely to ensue. As an extreme example may be mentioned a species of nemertean of which two individuals, each more than a foot in length, were found on the shore at Woods Hole, Mass., during an extremely low tide in June, 1894. Their morphological peculiarities were such as to make them the type

of a new genus (Parapolia). In spite of persistent search in the same locality and elsewhere during many of the intervening years, no additional representatives of the species have been found. And yet it seems not improbable that numerous individuals are living somewhere in the vicinity, perhaps somewhat below the low-water mark, for in such situations burrowing animals are difficult to obtain.

It may be assumed therefore that "Polia or Eupolia," which is mentioned by Wheeler as the possible "mutation mother-species" of the form with branched proboscis, is not "moribund" and it may be confidently expected that it will appear again in full vigor and quite unchanged by the allegation that it is at present masquerading in the guise of Gorgonorhynchus.

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The Deterioration of Rubber Products by Micro-Organisms

By Claude E. ZoBell and Josephine D. Beckwith

ALTHOUGH rubber is generally regarded as being relatively inert, it is slowly attacked by certain micro-organisms. This first came to the attention of the senior author when, due to a shortage of glass-stoppered bottles, an attempt was made to supplement them with rubber-stoppered bottles for studying the B.O.D. of sea water. Almost invariably it was found that more oxygen was consumed by micro-organisms in sea water in the presence of rubber than in its absence. Even the small amount of rubber gasket exposed to the water in citrate of magnesia bottles caused an increase in the amount of oxygen consumed after 5 to 10 days incubation at 25°C. The examination of heat-sterilized and chemically preserved controls indicated that the majority of the increased oxygen consumption was caused by biological activity.

An extension of these observations and a precursory review of the literature reveals that various kinds of natural and synthetic rubber are attacked by numerous microbial species representing several genera.

A paper presented on October 29, 1943, at the California Section Meeting, Los Angeles, Calif., by Claude E. ZoBell, and Miss Josephine D. Beckwith, Scripps Inst. of Oceanography, Univ. of Calif., La Jolla, Calif.

Kinds of Micro-organisms Which Attack Rubber

Arens (2) attributed the red spots which appear on freshly prepared, unpreserved sheet rubber to the growth of *Bacterium prodigiosum*. Once established in the drying shed, this organism was found to cause considerable damage to sheet rubber. From red, blue and black spots on crude rubber, de Wilderman (12) isolated *Micrococcus prodiogiosus* and other chromogenic bacteria. The "spot disease" of crude sheet rubber exposed to moisture caused by the development of chromogenic organisms has been described by Eaton (14).

Blommendaal (5) demonstrated micrococcoid slime bacteria on crepe rubber showing white spots which appeared when the rubber was stored in the presence of air and moisture. Unlike mildews which commonly grow on the surface of crepe rubber, probably primarily at the expense of the nitrogenous impurities in the crude rubber, the "spot disease" bacteria are claimed to affect the usability of the rubber. Stevens (25) proposed the addition of 1.0 per cent p-nitrophenol to latex to prevent the growth of molds and other spot-forming organisms on crepe rubber.

Söhngen and Fol (21) observed the growth of certain soil micro-organisms on purified rubber treated with a min-

eral solution. *Actinomyces fuscus, Act. alba, Act. chromogens, Act. elastica* and another unnamed species of *Actinomyces* grew well in the mineral medium which contained only purified rubber as a source of energy. Fair growth was exhibited by *Mycobacterium rubrum, Mycobact. lacticola, Bacillus mesentericus* and *Pseudomonas fluorescens.* These organisms were isolated from garden soil and canal water by Söhngen and Fol.

According to de Vries (10, 11), the slow development of *Penicillium* and *Aspergillus* species on moist sheets of plantation crepe, which contain an average of 93 per cent of rubber hydrocarbons, causes a gradual loss in weight and physical properties of the rubber. The loss in weight was found to be as much as 22 per cent in 2 yr. and 30 per cent in 5 yr. The viscosity and elasticity of crepe rubber were markedly decreased.

From fermenting *Hevea* latex Corbet (1930) isolated *Gaffkya verneti, Micrococcus eatoni, M. ridleyi, M. epimetheus, M. chersonesia, Alcaligenes denieri, Bacillus pandora* and *Torulae heveae.* They discolored the latex and caused a slightly acid reaction which was shown not to be due to the fermentation of the methyl-1-inositol in the latex. Since there is no other substance present in latex except rubber hydrocarbon in sufficient quantities to account for the observed changes, Corbet concluded that the rubber hydrocarbon is attacked by the micro-organisms.

In earlier studies Corbet (7) found that heat-sterilized *Hevea* latex does not coagulate, which he attributed partly to the occurrence of thermolabile enzymes in the latex and partly to the action of bacteria. The inoculation of heat-sterilized latex with appropriate bacterial cultures resulted in coagulation after a short period of incubation. The bacterial count was described as tremendous in all cases when coagulation began. Predominating was *Bacillus pandora,* which attacked the coagulated rubber.

Novorgrudski (30) described a small non-motile coccus which forms yellow spots on rubber. These organisms, which are widespread in soil, decompose rubber and reduce the viscosity of the remaining rubber.

The decomposition of guayule latex by soil micro-organisms and by pure cultures of *Actinomyces* has been reported by Spence and McCallum (23) who found that 15 per cent of the rubber hydrocarbon disappeared and the remaining rubber had deteriorated in six weeks at 37°C. This observation was used to explain in part why there have been many failures to find appreciable quantities of rubber in guayule under certain conditions.

Besides finding four species of *Actinomyces* which decompose *Hevea* latex, Spence (22) found several strains of anaerobic bacteria which decompose the rubber in guayule latex.

Working with *Hevea* latex, Spence and van Niel (24) found that "the decomposition of rubber hydrocarbon by certain bacteria is both rapid and profound." After a few weeks at 30°C. the greater part of the rubber originally present in the latex had vanished, leaving a coagulum composed of a mass of bacteria in a residue bearing no resemblance to rubber. They found that after six weeks incubation at 20°C. soil organisms completely decomposed more than 20 per cent of the rubber in highly purified latex, "and that which remained was thoroughly deteriorated, soft and 'dead.'" Four species of *Actinomyces* were pri-

marily responsible for the deterioration of the rubber. These workers suggest that the products resulting from the microbial decomposition of natural rubber may provide a key to the study of how plants synthesize rubber.

Kalinenko (19) noted the destruction of rubber by *Actinomyces longisporus ruber, Act. aurantiacus, Aspergillus oryzae* and *Penicillium* species, which destroyed from 20 to 40 per cent of the rubber dispersed in mineral solution. *Azotobacter chroococcum, Bacillus mycoides, B. fluorescens, Sarcina ureae* and *Proteus vulgaris* were unable to assimilate the rubber hydrocarbon.

Bigger and Nelson (4) substantiated Harold's observation that the presence of rubber rendered distilled water growth-promoting for certain coliform bacteria. Questioning the ability of bacteria to assimilate rubber, these workers attributed the growth-promoting properties of rubber tubing and rubber stoppers to the talc dressing which they believed adsorbed nutrients from the atmosphere.

Using oxygen consumption, carbon dioxide production, the multiplication of micro-organisms and the deterioration of rubber as criteria, ZoBell and Grant (27) noted the oxidation of various kinds of natural and synthetic rubber by species of *Actinomyces, Micromonospora, Proactinomyces, Mycobacterium* and *Pseudomonas* which are quite widely distributed in the sea and in garden soil. Although they presented no data to prove that microbial activity plays an important role in the deterioration of rubber products, ZoBell and Grant concluded from their observations that the life of rubber products which remain in contact with moisture may be prolonged by retarding or preventing the activities of rubber-oxidizing micro-organisms.

Micro-organisms which oxidize rubber appear to be widely distributed in nature. Such organisms are almost invariably found associated with unpreserved latex and rubber products exposed to moisture. Old pieces of garden hose and the cracks in rubber products left in moist places have proved to be good sources of cultures.

Neither in nature nor in the laboratory do rubber-oxidizing bacteria appear to require rubber hydrocarbon for their growth. Though most abundant on moist rubber, rubber-oxidizing bacteria have been demonstrated in nearly all samples of marine mud, sea water, sewage, tap water and soil which have been examined for their presence. The enrichment culture technic has been employed to demonstrate the presence of rubber-oxidizing bacteria in such materials.

Following the method outlined by Kalinenko (19), garden soil has been found to contain from 5 to 500 rubber-oxidizing micro-organisms per gram (dry basis). Mineral-salts agar plates are coated with a thin film of rubber by allowing a benzene solution of purified rubber to evaporate on the surface of the agar. Included with the mineral salts is 0.01 per cent ammonium phosphate to provide for the nitrogen requirements of the organisms. The prepared plates are sprinkled with a small quantity of finely pulverized soil. After a week or two incubation at 25°C., the increasing transparency of the agar plates followed by the gradual perforation of the rubber film indicates the presence of rubber-oxidizing colonies. Various species of *Actinomyces* and *Proactinomyces* predominate on such plates with smaller numbers of *Micromonospora, Mycobacterium, Pseudomonas, Bacillus* and probably other genera.

Judging from the results obtained with two dozen species of soil and water bacteria selected at random from our stock cultures, several bacteria have the ability to attack rubber hydrocarbon. Mineral solution, including ammonium phosphate containing purified rubber as the only source of energy and organic matter, was inoculated in 150-ml. glass-stoppered bottles. Table 1 shows the amount of oxygen consumed by these cultures in 10 days at 26°C. in the presence of rubber after making corrections for their endogenous respiration in the same mineral solution without rubber. The bacteria were unable to multiply in the control mineral solution to which no rubber was added. All cultures were incubated in the dark.

TABLE 1

Oxygen Consumed by Bacteria Growing in Mineral Solution From the Oxidation of Purified Rubber

Culture	O_2 consumed—ppm.	Culture—Cont.	O_2 consumed—ppm.
None (sterile control)	0.30	Escherichia coli (SIO)	3.64
Pseudomonas neritica	5.23	Escherichia coli (R)	0.40
Pseudomonas aliphaticum	7.85	Serratia marcescens	1.99
Pseudomonas fluorescens	7.04	Serratia marinorubra	0.33
Pseudomonas sp. H-34	7.76	Alkaligenes fecalis	0.78
Bacillus subtilis	2.81	Sarcina lutea	0.20
Bacillus megatherium	0.17	Sarcina pelagia	0.37
Vibrio marinofulvus	5.35	Proteus vulgaris	0.40
Achromobacter stationis	0.29	Staphylococcus citreus	0.20
Flavobacterium marinovirosum	0.53	Micrococcus sedentarius	0.26

Since the rubber in the presence of 150 ml. of the mineral solution abiogenically consumed an average of 0.30 ppm. of oxygen under the experimental conditions, and since there may be an inherent experimental error of as much as 0.20 ppm. in determining the oxygen uptake, only the consistent consumption of more than 0.50 ppm. of oxygen is regarded as evidence of biological activity. Certain strains of *Escherichia coli* developed slowly, utilizing rubber as the only source of energy, but other strains failed to develop in such a medium.

Natural Rubber

Perfectly pure rubber, also called caoutchouc, is an unsaturated hydrocarbon having the composition $(C_5H_8)_n$. Caoutchouc is produced by a wide variety of plants, the best yields being obtained from the families Euphorbiaceae, Moraceae and Apocyanceae. Commercially, rubber is obtained chiefly from the plantation rubber tree, *Hevea brasiliensis*. Lesser quantities are obtained from *Hevea benthamiana, Castilla ulei, Castilla elastica, Manihot glaziovii, Fiscus elastica, Funtumia elastica* and other wild rubber trees. High percentages of caoutchouc also occur in the guayule plant, *Parthenium argentatum,* certain milkweeds, *Asclepias* species, the Russian dandelion, *Taraxicum kok-saghyz* and the Haitian shrub, *Cryptostegia grandiflora.* Caoutchouc occurs in many other plants.

The fact that rubber or caoutchouc is produced by numerous plants, and yet never accumulates in nature, indicates that it must undergo decomposition. Actually, pure rubber is relatively unstable, being oxidizable in the presence of atmospheric oxygen, particularly when exposed to light. The oxidation of rubber appears to occur most rapidly in environments where conditions are conducive to the growth of micro-organisms. The microbial deterioration of *Hevea* latex, which contains from 30 to 40 per cent rubber, is prevented by the addition of ammonia, caustic soda or other preservatives. Without such preservatives the latex ferments, resulting in the gradual destruction of the rubber hydrocarbon (8, 24). The microbial destruction of guayule latex and crepe rubber has been reported by Spence and McCallum (23) and de Vries (11) respectively.

When *Hevea* latex is cured by drying or acid coagulation, the resulting product contains from 90 to 95 per cent rubber. Air- and smoke-dried latex contains a much higher percentage of impurities than plantation crepe or sheet rubber which is acid coagulated. The average content of impurities of rubber prepared by the two methods is given below as compiled from various reference books:

Constituent	Air- or smoke-dried latex— Percentage	Acid-coagulated crepe rubber— Percentage
Nitrogenous materials	4 to 6	1 to 3
Acetone-soluble constituents	3 to 5	2 to 3
Mineral matter or ash	1 to 2	0.2 to 0.4
Methyl-l-inositol	1 to 2	traces
Sugars	0.3 to 0.5	traces

The acetone-soluble constituents consist primarily of stearic acid, oleic acid and various sterol-like compounds. From these data it is evident that the mere growth of micro-organisms on the surface of dried latex or crepe rubber is not conclusive proof that the rubber itself is being attacked because there are ample impurities to provide for bacterial multiplication. However, experiments with highly purified rubber, as well as with manufactured rubber articles, show that both pure rubber and compounded rubber articles are attacked by micro-organisms.

Compounding Ingredients

Manufactured rubber articles are prepared by compounding natural or synthetic rubber used singly or blended together in different proportions with hundreds of material to give certain desirable properties of elasticity, resiliency, extensibility, vulcanizability, tensile strength, conductivity, color wearing qualities, heat resistance, inertness towards solvents, etc. The commonest of the compounding ingredients may be classified in the following categories:

(a) Vulcanizing agents, of which sulfur is most important. Soft rubber generally contains from 1 to 10 per cent sulfur and hard rubber may contain from 10 to 50 per cent sulfur. Sulfur monochloride, selenium and other substances are sometimes used as vulcanizing agents.

(b) Accelerators of vulcanization, consisting of such substances as diphenylguanidine, *p*-aminodimethylanaline, thiocarbanilid, mercaptobenzothiazole (known to the rubber industry as Captax) tetramethylthiuram disulfide (known as Tuads), di-ortho-tolylguanidine dicatechol borate (Permalux) and other organic amines used singly or in combination. Compounded rubber generally contains from 1 to 5 per cent

accelerator. According to Dimond and Horsfall (13), Tuads and Captax are bacteriostatic, but whether they prevent bacterial activity when compounded with rubber is still unknown.

(c) Resistors, inhibitors or antioxidants, which consist of 0.5 to 4 per cent of para-amino-phenol (Rodinal), phenyl-alpha-naphthylamine (Neozone), diphenylamino ethane, certain aldehyde-amines or other compounds. While these substances retard the autoxidation of rubber and while some of the antioxidants are germicides, they may not inhibit the growth of micro-organisms in the form and concentration in which they occur in compounded rubber.

(d) As softeners, various hydrocarbon oils, waxes, tars, resins, fatty acids (usually stearic) and other substances are employed in concentrations ranging from 0.2 to 5 per cent. Larger quantities of fatice, or rubber substitutes made by treating vegetable oils with sulfur or sulfur chloride, are sometimes used as softeners or fillers.

(e) Reinforcing pigments or fillers consist of 1 to 60 per cent of one or more of the following: zinc oxide, carbon black, magnesium carbonate, lithopone, ferric oxide, zinc chromate, titanium oxide, lead oxide, certain clays, calcium carbonate, ground slate, metals, balata, gutta percha, fabrics, wood, etc. Wild rubber and low-grade plantation rubber sometimes contains sand, bark, leaf fragments and other impurities.

(f) Antimicrobial agents or antiseptics are only occasionally considered. Chlorothymol, p-nitrophenol, chlorinated dihydroxydiphenylmethane, sodium pentachlorophenate, mercurials and cyanides are among the long list of substances which have been used experimentally for this purpose. Manufacturers are giving more and more attention to the development of antimicrobial rubber for products continuously exposed to water.

Additional information on the composition of compounded rubber is given by Davis and Blake (9), Memmler (20), Fisher (16), Barron (3) and Flint (17).

In our initial experiments highly purified, 99 + per cent, non-vulcanized rubber obtained from the Goodyear Tire and Rubber Co., was dissolved in cp. benzene. After permitting the fractions insoluble in benzene to settle out, enough of the supernatant solution to contain 0.3 mg. of rubber was transferred to 150-ml. glass-stoppered bottles which were manipulated to form a thin film of rubber on the inside of the bottles as the benzene evaporated. After expelling the last trace of benzene, the rubber-coated bottles were filled with sterile water. Some of the bottles were inoculated with bacterial cultures and some were left uninoculated as sterile controls. Duplicate bottles were examined at the beginning of the experiment for bacteria and oxygen content, and other bottles were examined after different periods of incubation in a water bath at 22°C. The cultures were incubated in the dark. The protocol of a representative experiment is given in Table 2.

The oxygen content of the water was determined by a modified Winkler technic. Since iodine reacts with rubber, the water from each 150-ml. bottle was carefully siphoned into two 60-ml. bottles prior to introducing the Winkler reagents, from which iodine is liberated in the presence of oxygen.

A progressive increase in the amount of oxygen consumed was noted in the inoculated water in the presence of rubber, which was appreciably more than the amount of oxygen consumed by cultures without rubber or by rubber

TABLE 2

Oxygen Content of Sterile and Inoculated Mineral Solution, Some of Which Was in the Presence of the Equivalent of 2 mg. of Highly Purified Rubber per Liter, After Different Periods of Incubation at 22° C.

Experimental conditions	Oxygen content of water after—ppm.				O_2 consumed in 10 days—ppm.
	0 days	2 days	5 days	10 days	
No rubber, sterile control	6.86	6.88	6.85	6.87	0
No rubber, inoculated	6.80	6.72	6.64	6.51	0.29
With rubber, sterile control	6.83	6.52	6.30	6.29	0.54
With rubber, inoculated	6.79	5.47	2.31	0.82	5.97

in uninoculated water. After 10 days the equivalent of 5.14 ppm. of oxygen was consumed due to the action of bacteria on 2.0 ppm. of rubber (0.3 mg. of rubber per 150 ml. of water equals 2.0 ppm.). The quantitative oxidation of 2 mg. of rubber to carbon dioxide and water would require 6.58 mg. of oxygen, assuming rubber to be $(C_5H_8)_n$. Since 5.14 mg. of oxygen was consumed in the presence of 2.0 mg. of rubber, it is calculated that 78 per cent of the rubber was oxidized to carbon dioxide and water. Determination of the carbon dioxide content of the water substantiated this conclusion.

Oxygen consumption was accompanied by the multiplication of bacteria as shown by plate counts and the direct microscopic observation of samples. From the mass of bacteria produced it is estimated that 10 to 20 per cent of the rubber was converted into bacterial protoplasm. When added to the amount oxidized, it is found that the purified rubber is almost quantitatively utilized by bacteria, thereby confirming the observation of Spence and van Niel (24) that rubber hydrocarbon is completely decomposed by certain micro-organisms.

The perforation followed by the gradual and complete disappearance of thin films of rubber on inoculated agar plates and on glass slides immersed in culture solutions gives further evidence that rubber is attacked by micro-organisms.

Extensive Experiments

Experiments similar to the one summarized in Table 2 have been extended to include various micro-organisms both mixed and pure cultures from different sources employing several different kinds of manufactured rubber articles. The rubber articles were manufactured from natural rubber, reclaimed rubber and synthetic rubber.

Pure rubber is oxidized more rapidly by bacteria than most compounded rubber products. This is illustrated by the data in Table 3 which were obtained by exposing different rubber products to bacterial cultures in 150-ml. glass-stoppered bottles. The rubber products were cut into small pieces of approximately equal size to provide for the exposure of equal areas of rubber surface, because it has been found that the rate of oxidation is directly proportional to surface area rather than the weight of rubber. The amount of rubber in rubber products varies within wide limits depending upon their content of compounding agents.

Pure rubber or caoutchouc may be

oxidized more readily than compounded rubber either because in the latter the hydrocarbon molecules are bound with the compounding agents in such a way that they are less susceptible to attack, or some of the compounding agents may be bacteriostatic. The problem of anti-microbial properties of compounding agents cannot be considered in this paper which is concerned primarily with establishing that under conditions conducive to the growth of micro-organisms, rubber products are subject to microbial attack.

The action of bacteria on the inner lining of garden hoses is believed to be one of the chief causes of their failure. The lining usually consists of natural or reclaimed rubber compounded with various fillers. Judging from the rate at which small pieces of the lining of intermediate grade (pre-war, guaranteed for 5 yr.) garden hose are decomposed by bacteria in water in controlled experiments, it is estimated that as much as 1.0 g. of rubber may be oxidized per day in a 50-ft. length of hose left full of water at 22°C. In a year this would amount to more than ½ lb. of rubber under optimal conditions for the activity of micro-organisms.

Besides the loss of rubber, bacterial attack causes rubber to swell and it probably weakens the structure of the rubber product. Fortunately, conditions are rarely optimal for bacterial activity in garden hoses. Oftentimes they are drained, or the hose may be left only partly filled with water. During the time that water under pressure is flowing through the hose, bacterial activity will be minimized. When manufactured from an abundant supply of cheap rubber, the bacterial deterioration of garden hoses may be of no economic importance but when the stock-piles of rubber are rapidly diminishing, the problem of the bacterial deterioration of rubber may merit consideration.

Motivated by his interest in the early failure of rubber products used in water distribution systems which are

TABLE 3

B.O.D. of Approximately 15 sq.cm. of Various Kinds of Rubber Products After 5 Days Incubation in 150 ml. of Mineral Solution at 22° C.

Rubber Product	Oxygen consumed in 5 days—*ppm.*		B.O.D. of Rubber Product—*ppm.*
	Sterile Control	With Bacteria	
None (mineral solution only)	0	0.31	—
Black rubber stoppers, "pure gum"	0.84	4.27	3.12
Amber rubber tubing, "pure gum"	0.72	5.14	4.11
Red rubber tubing, no fabric	0.53	5.80	4.96
White rubber tubing, no fabric	1.07	3.56	2.17
Rubber tissue, dental dam	0.41	5.09	4.27
Rubber gaskets, for mason jars	0.19	4.28	3.78
Rubber gaskets, for CM.* bottles	0.36	4.12	3.45
Rubber lining from garden hose	0.42	5.98	5.25
Purified caoutchouc, 99+ per cent rubber	0.45	6.82	6.06

* CM is used to abbreviate citrate of magnesium bottles.

costly to service, Carl Wilson, consulting technologist in water supply, provided us with some rubber rings or gaskets used in connecting asbestos cement pipe, rubber gaskets used in water meters and a hard rubber plate or disc used in water meters. The effect of bacteria upon these products is summarized by the data in Table 4.

The mere utilization of oxygen in the presence of rubber products does not necessarily prove that rubber itself is oxidized but when corrections

TABLE 4

Oxygen Consumed in 10 Days by Comparable Surface Areas of Different Rubber Products in Sterile Mineral Solution and in a Similar Solution Inoculated With Bacterial Cultures

Rubber Product	Oxygen Consumed in 10 Days—*ppm.*		
	Sterile water	Culture No. 43	Culture No. 87
None (mineral solution only)	0	0.40	0.34
Gaskets for asbestos cement pipe	0.45	3.99	4.73
Water meter gasket	0.55	5.16	6.77
Hard rubber water meter plate	0.48	1.07	1.32
Neoprene rubber stopper	0.63	5.17	3.46

are made for the controls, the B.O.D. is indicative of the quantity of rubber product that has been oxidized. For practical purposes it is inconsequential whether the rubber hydrocarbons or the compounding agents used in the manufacture of the rubber product are oxidized. Either would contribute to the deterioration of the product. Observations on the B.O.D. supplemented by data on the loss of weight of rubber products over a long period of time show that microbial activity accelerates the deterioration of rubber products five- to fifty-fold.

There is some evidence that the disagreeable taste imparted to water stored in rubber receptacles is intensified by microbial activity. Sterile water stored in contact with rubber tastes less bad than otherwise comparable water previously inoculated with rubber oxidizing micro-organisms.

An anonymous (1) editorial in a trade journal relates that the destruction of rubber insulation by fungi causes the failure of electric conduits. Various preservatives have been used to prevent the microbial deterioration of rubber insulating materials subjected to moisture.

Synthetic Rubber

The list of synthetic rubbers including Neoprene, Butaprene, Buna S, Buna N, Butyl, Thiokol, Perbunan, Hycar, Duprene, Chemigum, Agripol, Norepol, Flexon, Ameripol and Koroseal is being extended as new formulas are developed. Although the synthetic rubbers differ from each other in many respects, they all have certain properties in common with natural rubber. Most of them are polymers or co-polymers of unsaturated hydrocarbons such as butadiene ($CH_2=CH-CH=CH_2$), methylbutadiene or isoprene, chlorobutadiene or chloroprene, isobutylene ($(CH_3)_2C=CH_2$), acrylonitrile ($CH_2=CHCN$), styrene ($C_6H_5-CH=CH_2$), or allied compounds. Neoprenes are polymers of chloroprene. Buna-S types are co-polymers of butadiene and styrene. Buna-N types are co-polymers of butadiene and acrylonitrile. Butyl rubber is a co-polymer of isobutylene, butadiene and isoprene. Thiokol RD is a buna-N type of synthetic rubber. Thiokols A, B and FA made from ethylene chlo-

ride or dichloroethyl ether and sodium tetrasulfide differ markedly from the other synthetic rubbers. The other names mentioned above are trade names applied to synthetic rubber, most of them being buna-N types.

A priori one may expect the synthetic rubber to be attacked by bacteria because natural rubber is attacked and most kinds of hydrocarbons are oxidized by certain micro-organisms. According to Bushnell and Haas (6), Stone et al. (26), ZoBell et al. (28) and others, micro-organisms which attack hydrocarbons are widely distributed in water and soil. In general, unsaturated hydrocarbons are attacked more readily than saturated compounds, and long chains are attacked more readily than short chains. The oxidizability also seems to be a function of the number of side-chains, iso-compounds generally being more susceptible to bacterial oxidation than normal hydrocarbons. Most synthetic rubbers are long-chain unsaturated hydrocarbons with many side chains attached.

Most uncured synthetic rubbers are very unstable, and even when compounded with resistors or antioxidants they appear to be susceptible to bacterial attack. Table 5 illustrates the effect of bacterial activity on a series of synthetic rubbers provided by the United States Rubber Co. A sample of highly purified, 99 + per cent, natural rubber and one Neoprene (rubber stopper) are included for comparison. The rubber was distributed in glass-stoppered bottles so that approximately equal areas of each were exposed to the water. Sea water diluted with distilled water was employed to provide the minerals essential for the growth of micro-organisms. Part of the bottles were inoculated with enrichment culture No. 43 isolated from sea water, part were inoculated with culture No. 87 isolated from fresh water and part were left uninoculated as sterile controls. The oxygen content of the water was determined in duplicate at the beginning of the experiment and after 10 days incubation at 26°C. The amount of oxygen consumed by the rubber in the presence and in the absence of bacteria is summarized in Table 5.

TABLE 5

Oxygen Consumed in 10 Days at 26°C. by Comparable Surface Areas of Different Kinds of Rubber in Sterile Mineral Solution and in a Similar Solution Inoculated With Bacterial Cultures

Description of rubber	Oxygen Consumed in 10 Days—*ppm.*		
	Sterile control	Culture No. 43	Culture No. 87
None (mineral solution only)	0	0.40	0.58
Buna S (butadiene and styrene type)	0.70	5.69	6.28
Thiokol (organic polysulfide type)	0.62	2.22	3.34
Hycar (Buna N type)	0.82	3.90	3.28
Butyl (Isobutylene co-polymer type)	0.77	1.95	2.06
Experimental elastomer 8-D	0.67	2.54	1.69
Experimental elastomer 8-E	0.65	1.94	2.90
Experimental elastomer 8-F	1.45	2.24	4.84
Neoprene (du Pont stopper)	0.97	5.17	3.46
Purified natural rubber	0.47	6.62	7.48

Multiple Formulas

The data in Table 5 indicate that bacteria promote the oxidation of various kinds of synthetic rubber. However, such data are of no significance for appraising the relative resistance of a general type of rubber to oxidation without information on the formulas used in compounding the particular product, there being literally hundreds

of formulas for giving the rubber products certain desirable properties.

Further experiments employing oxygen consumption as well as changes in the weight and consistency of the rubber itself show that rubber products manufactured from Neoprene, Duprene, Butaprene, Chemigum and Ameripol are attacked by micro-organisms in mineral solutions. Although we do not have the formulas used in compounding the synthetic rubber products which were examined, we understand that they contained no natural or reclaimed rubber. In these experiments which were designed to show whether a given product is subject to microbial attack rather than to obtain data on the relative rates of biological decomposition of different rubber products, most kinds of synthetic rubber products, including laboratory appliances such as stoppers and tubing, appeared to be equally, or possibly more, susceptible to oxidation by bacteria than products manufactured from natural rubbers. Polysulfide thiokols (types A and B), vinylites and chlorinated rubbers appear to be relatively resistant to bacterial attack but these products have few of the characteristics of live rubber.

Discussion

The foregoing observations indicate that the anti-microbial properties of rubber products should be taken into consideration in the selection or development of rubber which is to be exposed continuously to moisture under conditions favorable for the growth of micro-organisms. This is not a simple problem. Substances which are normally bacteriostatic may be biologically inactive when combined with rubber hydrocarbons or rubber compounding agents. For example, Dimond and Horsfall (13) found that while mercaptobenzothiazole is a potent anti-microbial agent by itself, it is rendered ineffective as an inhibitor of micro-organisms when mixed with zinc oxide which is commonly used in compounding rubber products. On the other hand certain bacteriostatic agents may impart undesirable properties to the rubber.

Besides the technological difficulties which are involved, the problem of preventing the bacterial decomposition of rubber hydrocarbons is complicated by the fact that a good many cyclic or aromatic compounds of the benzene series which are commonly used as germicides or preservatives are attacked by some of the hydrocarbon-oxidizing bacteria. Gray and Thornton (18) found bacteria to be quite widely distributed in the soil, which oxidize phenol, cresol, phloroglucinol, toluol and recorcinol. Erikson (15) studied *Micromonospora* species from mud, which utilize toluol, phenol, resorcinol, cresol, β-naphthol, naphthalene and paradichlorobenzene. ZoBell et al. (28) have observed the growth or water bacteria in the presence of 1.0 per cent phenol and a like concentration of emulsified tri-cresol.

The problem of the role of micro-organisms in the deterioration of rubber products and its prevention has received relatively little attention although considerable work has been done on the microbiology of *Hevea* latex and crude crepe rubber. This is because latex and crepe rubber are subjected to conditions which favor microbial activity, while proportionally only small quantities of manufactured rubber products are exposed to environments where appreciable microbial activity is possible. The extensive growth of micro-organisms on rubber

has been observed only in the presence of moisture at a favorable pH, temperature and oxygen tension. For most rubber products the effect of microbial oxidation is either non-existent or insignificant compared with the deterioration caused by heat, light, oil, abrasion, air, acid, alkali, etc.

Even in the presence of biologically balanced mineral solution containing available nitrogen, rubber is only slowly attacked by micro-organisms. In the experiments summarized on the preceding pages, from 0.2 to 0.5 mg. of rubber was oxidized in 10 days at 25°C. by active cultures growing on around 250 to 300 mg. of rubber cut into small pieces so that about 15 sq.cm. of surface area was exposed. At this rate the micro-organisms would decompose from 3 to 10 per cent of the rubber initially present in a year, or 5 to 50 times as much as undergoes spontaneous or abiogenic deterioration in a mineral solution during a like period. de Vries (10) noted a 22 per cent loss in weight in crude crepe rubber stored in the presence of moisture for 2 yr. and a 30 per cent loss in 5 yr. which he attributed to the activities of micro-organisms.

It is difficult to generalize concerning the percentage decomposition of rubber because the rate of bacterial oxidation is directly proportional to the surface area exposed to active cultures and bears little relationship to the total quantity of rubber present. Moreover, the rate of oxidation increases progressively from day to day after the organisms have started to grow on the surface of the rubber until a slimy film is established. Later in a closed system when D.O. is depleted from the surrounding water, bacterial oxidation ceases.

The oxygen tension of the water does not influence the rate of bacterial oxidation until the oxygen is nearly depleted. However, the bacterial as well as the abiogenic oxidation of rubber is influenced by the oxidation-reduction potential of the medium. After the oxygen content is reduced to less than 0.3 ppm., oxygen may be consumed by respiring bacteria on the surface of oxidizable substrates more rapidly than it can be replaced by diffusion, under which conditions the oxygen tension becomes a limiting factor. Using a variety of substrates, ZoBell and Stadler (29) demonstrated that the respiration of water bacteria is independent of oxygen tension within the examined ranges of 0.30 to 36.46 ppm.

Summary

Rubber hydrocarbon is attacked by a large number of micro-organisms including species of *Actinomyces, Proactinomyces, Micromonospora, Mycobacterium, Aspergillus, Penicillium, Pseudomonas, Bacillus* and other genera. Rubber is decomposed more rapidly by mixed cultures than by pure cultures.

Oxygen consumption, carbon dioxide production, the multiplication of micro-organisms and loss in weight of the rubber show that the rubber is being attacked in a medium which has no other source of carbon or energy. Microbial activity also causes rubber to swell.

Pure natural rubber or caoutchouc is more susceptible to oxidation by micro-organisms than compounded rubber products although many rubber products are attacked.

Microbial activity may play an important role in the deterioration of rubber products which are exposed to moisture under conditions favorable

for the growth of micro-organisms. Certain rubber products used in water distribution systems are subject to microbial attack. From experimental data it is estimated that up to one-half pound of rubber from the inner lining of a 50-ft. length of garden hose may be oxidized in a year if water is left standing in the hose.

Some synthetic rubbers are just as susceptible to microbial oxidation as natural rubber while others appear to be relatively resistant to microbial attack.

It is recommended that the antimicrobial properties of rubber be taken into consideration in the selection or development of rubber products which are to be continuously exposed to moisture.

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Discussion

Harry E. Jordan*

The authors of this paper have recorded a series of studies of considerable interest. Much of the material presented relates to the extent of bacterial growth on *crude* rubber. As a record of the susceptibility of this vegetable derivative to bacterial action, the information is valuable for anyone who has need for fundamental knowledge of the nature of rubber.

When the authors refer to studies upon compounded commercial rubber products, one gains the impression that the data presented might produce substantially erroneous impressions. With this statement of the authors there can be no disagreement—"the anti-microbial properties of rubber products should be taken into consideration in the selection or development of rubber which is to be exposed continuously to moisture under conditions favorable for the growth of micro-organisms." One can parallel that statement with the suggestion that evidence is lacking that microbial deterioration of compounded rubber as used in water works practice is a problem of engineering or economic importance. In other words, rubber as used in meter discs and gaskets, as rings for mechanical couplings of water pipe, as valve discs or steam driven pumps, etc., has not shown a record of deterioration or failure sufficient to cause engineers to search for substitutes.

The deformation of meter discs by hot water backflow is a serious problem and thermally resistant substitutes for rubber have been sought. But the wasting away of meter discs in service has not been a problem as would be the case if microbial destruction were continuous or general.

Rubber rings under compression in mechanical joints on water lines have known service records of more than forty years with disintegration (which might be attributed to microbial action) unheard of in the water works field.

Water works engineers have learned much about the deterioration of ferrous metal as a result of bacterial action. But with the evidence of such destruction of ferrous metal, the engineers move toward its more adequate protection.

We must conclude that while the authors' studies indicate that microbial activity *could* promote the disintegration of rubber products as used in water works practice (meter discs, pump valves, pipe gaskets, etc.), other factors not demonstrated by the au-

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thors, evidently *limit or block off* bacterially induced deterioration.

There appears to be reason to apprehend that disintegration of cured or vulcanized rubber will neither cause the failure of a water line where rubber gaskets are used, nor the failure of a water meter to register where a rubber disc is the moving, measuring unit. In other words, what the authors have done is to open the door to further investigation by themselves, or by others, to show why, in practical experience, the deterioration which they postulate, does not occur.

Authors' Closure

Perhaps we failed to qualify sufficiently our experiments with rubber gaskets. Our thesis is that bacteria *do* attack pure rubber and rubber products. The evidence is convincing. However, one could easily get the wrong impression regarding the quantity of certain rubber products which may be oxidized by bacteria in the absence of more explicit information on certain experimental conditions.

In order to provide for considerable surface area, "the rubber products were cut into small pieces" (note descriptive sentence under Table 3). As a matter of fact, the rubber products were cut into such extremely small pieces that thousands of times as much surface area was exposed as is exposed by the whole rubber product. Unless considerable surface area of some of the compounded rubber products is exposed to the action of bacteria, no oxidation can be demonstrated by ordinary experimental procedures.

We believe that slow bacterial deterioration of such rubber products as we examined does occur although under practical conditions the rate of deterioration is very slow because relatively little surface is exposed. In view of the modern trend in the manufacture of synthetic rubber products, the possibilities outlined in our paper may merit attention.

Contribution No. 220

BEACH MARKINGS MADE BY SAND HOPPERS

BY

K. O. EMERY

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PLATE I, APRIL, 1944

BEACH MARKINGS MADE BY SAND HOPPERS

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ABSTRACT

Beach markings made by sand hoppers are very common on some California beaches. These have characteristics which may permit their recognition in ancient sands with consequent identification of the sand as a former marine beach deposit.

INTRODUCTION

The writer has observed characteristic beach markings made by sand hoppers, or sand fleas, on a number of beaches in California. The markings are especially well developed near San Diego where most of the beach sand consists of laminae of light colored quartz and feldspar alternating with laminae of dark colored heavy minerals, similar to California beach sands described by Thompson (1937). In some places at the upper part of the beach these materials form alternating layers several centimeters in thickness.

The beaches support an abundant population of sand hoppers, a type of amphipod which feeds largely on kelp washed ashore by the waves. During the day, the hoppers either conceal themselves under kelp, or hide in ready-made holes formed by wave action (Palmer, 1928), or dig their own burrows (Bryson, 1865; Reid, 1938; Schellenberg, 1928; Verwey, 1929). Where they make burrows, characteristic patterns are formed by the sand which is removed during excavation. These patterns are abundant only on beaches of fine to medium sand and have not been observed by the writer on beaches of very coarse sand. They occur chiefly on the upper part of the beach near the high tide mark.

DESCRIPTION OF MARKINGS

One marking found on all beaches examined resembles a small ant hill. It is made by one or more species of small hopper which makes a burrow about 2 mm. in diameter and 1 to 2 cm. deep. The sand which is removed forms a small mound about 2 cm. in diameter and 5 mm. high but not very distinctive.

Much more striking are markings formed by a larger hopper which reaches a length of 3 cm. There are several species of these large hoppers but *Orchestoidea californiana* is by far most common near San Diego. A number of specimens were kindly identified by Dr. Martin Johnson. This hopper, on digging a hole, throws the sand backwards with powerful kicks until the hole is about 1 cm. deep, then it turns and kicks sand out of the hole in a direction approximately 180° opposite from that in which the first sand was thrown. By repeated turning and throwing out of sand, the hopper forms two rays of sand, each of which may have a length of 10 to 25 cm. and a width of 3 to 6 cm. The hole is elliptical and 5 to 12 mm. in longest diameter. The depth is commonly 10 to 25 cm. Another hopper, *Talitrus saltator*, makes the same kind of marking (Schellenberg, 1928).

Where the beach consists of alternating light and dark colored layers, the sand encountered near the bottom of the burrow may have a different color from that at the surface, so that the rays very often consist of dark sand lying upon a light colored sand surface. Such rays are very apparent even to casual observers (Pl. I, fig. 1). Usually a number of burrows occur within a small area so that rays from adjacent burrows intersect, giving rise to a "chicken-track" sort of pattern (Pl. I, fig. 2).

Burrowing starts immediately after the tide has uncovered the beach, but the best rays are formed several hours later

PLATE I

FIG. 1.—Ray pattern made by sand hoppers. Dark colored heavy mineral sand thrown from the burrows lies on light colored quartz and feldspar sand. The area covered by the photograph is about 1.5 × 1.0 meters.

FIG. 2.—"Chicken-track" pattern developed by intersecting rays from many sand hopper burrows. Scale is given by heel print in foreground.

FIG. 3.—Truncated bases of sand domes formed above sand hopper burrows. Note that these are concentric rings of dark and light colored sand and not domes projecting above the general surface of the beach.

when the sand is dry enough so that the grains do not stick together in lumps. Exposure of the markings for periods of several days usually results in obliteration or burial of the markings by rays from newer burrows. As the tide again covers the beach, the markings are destroyed except possibly under favorable conditions which may allow their burial just as rain prints, tracks, and swash marks are preserved (Fairchild, 1901; Clarke, 1918).

SAND DOMES

As high tide approaches, the incoming waves wash higher and higher on the beach. Usually the rays are destroyed by the first few waves which cover them. These waves or others following them very often form sand domes over the tops of the burrows. The domes are initiated by a wave which covers the top of the burrow with a plug of wet sand. As the next wave washes over the beach, water percolates into the sides of the burrow, displacing the air of the hole. Because the cover of wet sand over the hole is more or less air-tight, the air becomes slightly compressed. The pressure is released either by blowing out part of the cover of sand or by lifting up a dome of sand, and forming an "air laccolith." Domes formed in this manner commonly range from 1 to 12 cm. in diameter, and 2 to 30 mm. high with a roof thickness of 2 to 30 mm. The next wave which washes over the dome usually destroys it, but by truncating it so that the upturned edges are bevelled. Where the dome roof consists of several alternating laminae of light and dark colored sand, the site of the dome is marked by concentric rings of alternating light and dark sand. Many such rings can be seen near the upper part of the beach reached by the tide (Pl. I, fig. 3). Other domes examined by the writer were formed over small holes made by expulsion of interstitial air of the sand by an advancing wave as described by Palmer (1928). Domes have been described elsewhere as the result of still different processes (Reade, 1884).

GEOLOGICAL SIGNIFICANCE

Sand hoppers belong to the suborder Gammaridea which includes the family Talitridae of which at least two genera are known to form the ray-type beach marking. The geological age of this family is unknown, but closely related amphipods have been reported present in Tertiary and possibly also in Silurian rocks (Zittel, 1885). Therefore, markings similar to those formed at the present time may be present in ancient sediments. Such markings should be supplementary indications of a marine beach environment because they require a temporary drying of the beach such as occurs between tides.

The sand domes which form above a burrow are probably ephemeral but their truncated bases could be preserved in ancient water laid sands. However, neither the domes nor their bases may be diagnostic of marine beach conditions for they may be formed by more than one process.

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Contribution No. 221

A SEAWEED GOES TO WAR

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New Series, No. 221

A SE

PRIVATE GELIDIUM CARTILAGINEUM is the principal American agarphyte, the agarweed of commerce. It grows in surf-beaten rocks and thrives in turbulent waters, is harvested commercially at a depth of 10 to 30 feet. A delicate fern-like red-purple seaweed, it has male, female, and asexual plants: this one is asexual, producing tetraspores.

IN 1917 THE UNITED STATES went to war; Private *Macrocystis* was drafted. In 1941 the United States went to war again: this time Private *Gelidium* answered the call. The macrocystis is generally known as "giant kelp" and is specifically *Macrocystis pyrifera*. The gelidium is known as "agarweed" and is specifically *Gelidium cartilagineum*. Both are natives of California, also living in South Africa and a few other places. Both belong to the same group of plants which we used contemptuously to call "seaweeds," but from which we are now deriving millions of dollars worth of various products.

During the last war the United States was badly in need of potash to fertilize her farms and victory gardens. The supply of this material used to come entirely from Germany. The giant kelp was drafted to supply the badly needed chemical and for the duration of the war it was the second important source of this vitally important substance in agriculture. At that time, *Macrocystis* also supplied this country with acetone and calcium acetate which were used in the manufacture of smokeless powders and in preparing dopes for airplanes.

In the present war there is no longer any shortage of potash or acetone, which are being supplied from sources other than the kelps. There is, however, another shortage which threatens to endanger the health of the entire nation. This is agar, a special seaweed product contributed by *Gelidium*.

Gelidium cartilagineum was unknown to American industry during the first World War, for the United States and Japan were then allies and the supply of the special *Gelidium* product, agar, from the O not endangered. The situati war is different. The aga from Japan which amount tween six and seven hund sand pounds annually was t off. The government had to the available agar in this c order that this critical mate be wisely employed in esse ters. Local production wa aged, and industry is now produce enough agar to meet essential demands.

The most important ser agar renders to mankind, in peace, is as a bacteriologica medium. To determine the pathogenic bacteria in a dis determine whether certain found in the water supply ar ous, they have to be cultivate preparation of serum and a for protection against disease ticular types of bacteria invo also to be cultivated. And, tically all cases of bacteriolo ture, the medium used is i agar gel to which are adde different chemicals to enco growth of these microbes. P dom realize that agar is an in ble tool that scientists and p use to guard against disease ness. An agar shortage, scie lieve, will have a drastic effe health of the whole nation, and servicemen alike.

Undoubtedly most person the following two questions it that this obscure seaweed agar is so indispensable in the ological culture work? A don't we develop substitute we do with everything else?

To answer the first we ha back some sixty-two years that time bacteriologists w using gelatin to cultivate crobes. There were, at le major difficulties in using medium, which bacteriologi unable to overcome. Firs pathogenic bacteria liquefy

ED GOES TO WAR

K. Tseng, of the Scripps Institution of Oceanography at La Jolla, tells the story of Pri-elidium, the seaweed that has gone to war. Originally imported in great quantities, ıpan, it is being grown and harvested commercially in California for its specific aids ar effort as it is used for culture media, impressions work, lubrication, and in other ways.

at the blood temperature at ese forms should be culti-atin was a fluid, not a solid. erequisite that the medium solid throughout the course tion.

y it occurred to a German , Frau Franny Eilshemius ıo incidentally was a native ca, that a certain vegetable lled "agar-agar" might be e culture medium instead of She had been using agar for er kitchen in the preparation ıd vegetable jellies. She sug-s to her husband, a bacteri-ho in turn passed the idea to ɔch, the great medical scien-is classical work on the tu-bacillus published in 1882, lly made known to the sci-entific world the advantages of using agar instead of gelatin. The great doctor, however, apparently did not realize that this discovery was going to help tremendously the advance of the science of bacteriology, and merely mentioned it casually in a short sentence in his publication. Anyway, from that time on, bacteriologists have been using agar almost exclu-sively in cultivating the pathogenic microbes.

Agar has several peculiar charac-teristics which make it most valuable as a culture medium for microbes. Contrary to the current belief of most laymen, agar does not serve as food for the bacteria. It is rather its inert-ness towards these microorganisms that makes it most useful in bacteri-ological culture work. That is, most bacteria, especially the pathogenic ones, are unable to utilize agar for food. The agar will thus remain solid throughout the culture of most bac-teria whereas gelatin will be "eaten" by most of them, forming a liquid.

Another important characteristic of this seaweed product is its high melting point. While a one and a half per cent solution of agar will set to a firm gel at about 43°C. the resulting gel, once set, will not melt until heated to about 95°C. Thus it could remain solid at 37°C., at which temperature gelatin is a fluid.

A DIVING BOAT engaged in agarweed harvesting. The fully dressed diver is descending the steps with a weed-basket in his left hand. Standing next to the diver and holding a rope is the life-line tender who sees to it that the diver gets what he wants from the boat by special signals, and that he returns safely. *(Courtesy of the Scientific Monthly.)*

Agar is also outstanding in its gelling power, which is almost ten times that of gelatin. Its ease of preparation, its relatively little water of syneresis, its reversibility, its neutrality in reaction, its transparency, its ability to withstand high pressure for a certain length of time without undergoing hydrolysis, all contribute to make agar gel the most valuable culture medium that science has ever known to date.

As to the second question it may be said that in the last few years several agar substitutes have been suggested. Unfortunately all of these have limited application and none could as yet take the place of agar. While the *Gelidium* agar still has its defects, so far as bacteriological work is concerned, it does not seem encouraging that we could find a better, cheaper and more easily handled agar substitute in the near future.

Besides serving as bacteriological medium, agar is also helping the war effort in various other ways. Owing to its long lag of temperatures between solidifying and melting, as pointed out above, it is used extensively in dental impression work. Its extraordinary water absorbing ability is utilized in wound dressing. Its extensive use as a laxative is similarly based on this ability to absorb and hold large amounts of water, becoming at the same time a lubricant and a mild stimulant. In a recent article in *The American Legion Magazine,* it is reported that agar is used to take the molds for making artificial hands for wounded service men.

Agar is also serving war-time industry in various capacities. For instance, in the hot drawing of tungsten wire, a lubricant is necessary to facilitate the drawing operations and to reduce the wear on the die. Westinghouse for years has been using a lubricant made of homogeneous suspension of powdered graphite in agar gel. It is used in the manufacture of marine storage batteries. In peace time agar is also extensively used in bakery, in liquid clarifying, in making health food, in confectionery, in ice cream, in silk sizing and in many, many different ways.

In its twenty years of existence the American agar industry has had its rise and fall. Strange to say, the person who first laid the foundation of the industry in this country, thus unconsciously freeing the United States from her dependence upon Japan for her supply of this vital article, was a Japanese, Chokichi Matsuoka. About 1920 he established the first agar factory in this hemisphere at Tropica (now Glendale), California. He secured a U. S. patent for his agar manufacturing process in 1921 and another one in 1923. It may be of some interest to note that in his first patent he emphasized the use of *Gloiopeltis* in his process and only casually mentioned *Gelidium* as a substitute. This was amusing because *Gloiopeltis* does not grow in quantity in California. In fact, it does not contain any appreciable amount of agar, and has never been used as an agar raw material elsewhere, Japan included. Evidently he actually used *Gelidium,* but, in order to cover this fact, he purposely emphasized *Gloiopeltis* as the raw material!

The original process of agar manufacture by Matsuoka was similar to the orthodox Japanese method, which involved too much human labor for an American industry. Probably because of this the Japanese company was not successful and a few years later was sold to a group of Americans. It remained for an obscure southern California inventor, John Becker, to modernize the whole process and lay the foundation of the present agar industry, which is doing so amicably in the present emergency. Becker took out several patents on his inventions. He improved the methods of cooking, filtering, congealing and bleaching and also invented the "dewatering" equipment and the sizing and dehydrating of agar flakes.

The agarweed *Gelidium c*
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directed to this goal.

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STUDIES OF THE VITALLY STAINED GOLGI APPARATUS

II. YOLK FORMATION AND PIGMENT CONCENTRATION IN THE MUSSEL MYTILUS CALIFORNIANUS CONRAD

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ONE TEXT FIGURE AND THREE PLATES (EIGHTEEN FIGURES)

INTRODUCTION

The eggs of animals, at ovulation, are endowed with reserve food stores in the form of carbohydrate, fat and protein. Typically, the carbohydrate (glycogen, galactogen, etc.) is diffusely distributed in the cell and is not incorporated into microscopically visible formed inclusions, but usually much of the fat and protein occur as granules, discs or spheres, thus constituting the yolk — fatty, or lipoidal, and albuminous, or protein. Yolk is laid down both during oögenesis and during the embryonic stages so that, although it is being consumed continually during development, the organism at hatching or at the attainment of the larval stage is still possessed of a considerable supply. In fact in some animals, notably fishes (Needham, '31), the embryo actually contains more fat at hatching than at fertilization.

Little study has been made of the morphology of yolk formation during embryonic development, but there have been numerous contributions dealing with this subject during oögenesis and, as is well known to cytologists, the problem has been subject to a wide variety of interpretation (see, for example, MacBride and Hewer, '31). At present, however, there appears to be fair agreement that, in animals generally, the lipoidal yolk arises in intimate association with the Golgi apparatus. On the other hand, the albuminous yolk, depending on the investigator, is traced to the nucleolus, to the mitochondria, to the Golgi elements, to the general cytoplasm or to a combination of two or more of these components. Almost invariably, authors have not traced both types of yolk to a common source. Thus, in those cases where the fatty yolk is reported to arise in association with the Golgi apparatus, the albumin-

[1] Contribution no. 51 from the Department of Biology, Brooklyn College.

[2] Contribution from the Scripps Institution of Oceanography, New Series, no. 222.

ous yolk has been traced to the nucleolus or to the mitochondria; in those cases where the Golgi apparatus is reported as the source of the albuminous yolk, the fatty yolk is said to arise from the nucleolus, or from a yolk nucleus, a vitelline layer, the general cytoplasm, etc. Recently, a few exceptions to the above statement have appeared. In the urchin Stomopneustes, Subramaniam and Gopola Aiyar ('36) state that the Golgi apparatus elaborates yolk of both types and Clement ('34) concluded that all of the yolk in the egg of the squirrel Sciurus, was a product of the Golgi material. In the latter case, however, it is not clear whether or not there is more than one type of yolk in the egg. Finally, Worley and Worley ('43), studying the problem in living material, noted that during the embryonic development of the tectibranch Navanax, the Golgi bodies discharged both fatty and protein yolk products.

In the present study, vitellogenesis has been investigated, both during oögenesis and in the living early embryonic stages of the California sea mussel, Mytilus californianus Conrad, a species representing another order of molluscs. The conditions in the pelecypod are compared with those found in the tectibranch and, incidentally, some observations have been made on the pigment absorbing and concentrating ability of the Golgi system.

The author is indebted to various members of the staff of the Scripps Institution of Oceanography and particularly to the Director of the laboratory and the Regents of the University of California for providing space and facilities for this work. I am pleased to acknowledge the advice and criticism of Prof. Wesley R. Coe in the interpretation of the observations and in the preparation of the manuscript. My thanks are due to Prof. Denis L. Fox for supplying the information on carotenoid pigment and to Elizabeth K. Worley for her work on the text figure.

METHODS

Ovaries and freshly spawned eggs of the mussel were treated with dilute methylene blue in sea water according to the method previously described ('43). Unexcised gonads were immersed in methylene blue for from 3 to 4 hours. They were then removed from the body of the animal, dissected on a slide and examined. Fertilized eggs were allowed to develop continuously in 1:250,000 methylene blue. Comparisons with unstained controls were continually made. The observations were repeated on six different batches of Mytilus eggs during March, April and May. Both stained and control specimens always

showed a small percentage of abnormal individuals which was no higher in the stained material, but which varied directly with the extent of crowding. Abnormal, injured or dying individuals usually showed a diffuse staining of the cytoplasm. Sometimes the nuclei were stained, but in the normal specimens, the stain was localized in the Golgi elements with hardly a trace of stain anywhere else. In Mytilus, the mitochondria were not observed to stain at all with methylene blue. In the normal embryos, no droplets of stain which could possibly be interpreted as "excretion droplets" formed in response to the presence of the vital dye, were ever noted. When the stain is properly employed on mollusc eggs, stained specimens and controls develop at the same rate and all live about the same length of time in the laboratory.

Neutral red was not used in this investigation because it was found in previous work that eggs would not develop normally in that stain. Methylene blue, therefore, is considered much more reliable, at least for this type of material, but it should be noted that not all invertebrate eggs can be reared successfully in methylene blue. With echinoderm eggs, for example, repeated trials have usually resulted in the failure of normal development during gastrulation, a condition that is correlated with a discrepancy in developmental rate between stained and control eggs. Brooks ('43) reports a similar experience with the eggs of echinoderms.

Permanent preparations of Mytilus eggs were made in which the following techniques were employed:

Regaud's fixative followed by aniline acid fuchsin and methyl green (Altmann)

Regaud's fixative followed by crystal violet (Benda)

Flemming's fluid with 1 drop of acetic per 5 cc. unstained and followed by iron hematoxylin

Kolatchev (McClung, '37, p. 276) unstained and followed by acid fuchsin

Mann-Kopsch (McClung, '37, p. 277) unstained and followed by acid fuchsin.

THE PRIMARY OÖCYTE

An adequate appreciation of yolk formation in Mytilus can be gained only after the structure of the primary oöcyte is understood and, for this reason, these observations take the freshly shed but uninseminated egg as a starting point. The primary oöcyte is 50–70μ in diameter. After discharge it becomes roughly spherical except for a slight depression at the animal pole, at which point the first maturation spindle reaches the surface. Endoplasm and cortex are sharply differentiated.

The egg is orange in color, especially when viewed en masse. This is due to a carotenoid pigment associated with the yolk spherules, but which is also diffusely distributed in the cytoplasm probably in association with fat droplets of ultramiscroscopic dimensions. The endoplasm is loaded with innumerable yolk granules about 1$^{\mu}$ in diameter (fig. 4). They are absent from the spindle area and egg cortex. Since these tiny spheres are pale yellow and because carotenoid pigment is known to occur almost always in association with fat or lipids, they are probably lipoidal in character. Furthermore, they turn brown when the fresh egg is treated with 2% osmic acid and they are black in Mann-Kopsch and Kolatchev preparations. In fresh material they show a positive reaction with Sudan III and they are about the same size as the known fat spheres of several other mollusc eggs. In the fixed and osmicated material, these fat granules are not always separable from the Golgi bodies, many of which are about the same size and shape, but the latter resist bleaching.

In many mollusc eggs, albuminous yolk spheres can be identified partly by their large size, but no such large inclusions occur in the Mytilus oöcyte, and after a considerable study of the egg, I am of the opinion that it contains no formed albuminous yolk. Millon's reagent turns the entire cell a pale rose without any localization of color. Rai ('30) reports the absence of protein yolk in the egg of another pelecypod, Ostrea. A casual examination of fixed material (not post-osmicated) stained with acid fuchsin or crystal violet, however, might lead one to identify the chromophobes of the simple vesicular Golgi bodies as albuminous yolk spheres, since both (in other eggs) stain similarly.

All of the positively identified mitochondria are rod-shaped, two to four times as long as wide. A few of these are scattered in the endoplasm, but the majority are lined up in enormous numbers perpendicular to the egg surface either within, or just below, the egg cortex. Their presence causes the cortex to stand out sharply in eggs stained with iron hematoxylin, acid fuchsin, or crystal violet (figs. 5 and 6, arrows).

The Golgi bodies are in the form of small (about 1$^{\mu}$) spheres or vesicles and minute chromophilic granules (under 1$^{\mu}$). These are the heavily stained bodies in the endoplasm in figures 5–7. When the egg is shed, some clumping of these elements is apparent (fig. 5). This soon disappears, however, as the bodies become more uniformly distributed due probably to cytoplasmic movement. After Regaud fixation followed by acid fuchsin or crystal violet, each Golgi sphere usually has a more or less uniform, homogeneous appearance. However, in living material stained with methylene blue, and frequently in fixed material stained

with iron hematoxylin, each Golgi sphere can be seen to consist of a chromophilic cortex and an eccentric, chromophobic center. The chromophilic cortex consequently appears thickened on one surface, forming a bowl-shaped "crescent" (fig. 1, f). Prolonged osmication blackens the cortex to such an extent that the vesicle appears as a black sphere without internal structure and insufficiently destained iron hematoxylin preparations present a similar picture. While this is inconvenient, it nevertheless offers good evidence that the chromophile completely encloses the chromophobe. The small Golgi granules appear homogeneous with all techniques and there is considerable evidence that they may not always blacken with osmic acid.

The similar behavior toward acid fuchsin, crystal violet and iron hematoxylin by the Golgi granules, by the chromophobes of the simple vesicular Golgi bodies and by the mitochondria and by the smaller, albuminous yolk spheres of other mollusc eggs, makes attempts at identification of these various elements a hazardous undertaking in fixed material. Supravital methylene blue staining goes a long way toward straightening out this difficulty. Yolk appears pale yellow-green, the Golgi elements are dark blue and the rod-shaped cortical mitochondria, which are so prominent in fixed preparations, are invisible. Since the only known mitochondria do not stain, it has been assumed partly on this ground that granules in the endoplasm which stain dark blue with methylene blue, are not mitochondria. This interpretation is further justified when it is observed in the fertilized egg that many of these dark blue granules enlarge and become Golgi bodies of the simple vesicular type. These granules must, therefore, be "reserve" Golgi elements and they have been so interpreted.

YOLK FORMATION DURING OÖGENESIS

Since the primary oöcyte is devoid of albuminous yolk, the yolk formation that occurs during oögenesis is probably largely fatty yolk elaboration. This fact is correlated with the high fat content of pelecypod eggs. According to Needham ('42), the iodine number for the egg of Pecten is higher than that of any other egg thus far investigated from this standpoint.

In the youngest Mytilus cells examined (very early oöcytes?, in fixed material only), the cytoplasm contains a few tiny chromophilic granules which are either Golgi granules or mitochondria, and clumps of small osmiophilic spheres which are undoubtedly simple vesicular Golgi bodies. By the time the cells have attained a diameter of 15μ (early oöcytes), these clumps have become broken up and the individual bodies

are fairly evenly distributed in the cell. Some of them by this stage have reached a diameter of over 1μ, but others probably exist as granules, since many granules are still present. Some of the mitochondria, now appearing as short rods, are lodged in the egg cortex.

The oöcytes of 25–30μ show peripheral rod-shaped mitochondria and, in addition to the types of Golgi bodies already mentioned, there occur large, spherical, vesicular forms 2–3μ in diameter (fig. 3, diagonal arrow). The diameter of the chromophobic vesicle in these particular bodies is two to three times greater than the diameter of the largest fatty yolk spheres. For this reason, as well as the fact that these Golgi elements are exactly like many of the protein-forming Golgi bodies of tectibranchs, they are considered protein forming here also. But it is apparent that, if this is the case, the protein that is elaborated undergoes dissolution in the cytoplasm during the late stages of oögenesis since no formed yolk inclusions of such size are to be found in the egg at the time of laying. Probably some of this protein (?) is consumed by the cell during this period and it is likewise possible that some of it, following dissolution, is converted into fat.

Hypertrophy of many of the Golgi bodies occurs as the oöcytes undergo further enlargement and there arise large puddingstone-like masses, 8–10μ in length by 4–5μ in width (fig. 2, horizontal arrow). A similar development that takes place in the post-gastrula stages is accomplished by the fusion of many somewhat hypertrophied small Golgi bodies. It is probable that a similar behavior occurs during oögenesis, since accompanying their increase in size, they become much less numerous. These compound vesicular Golgi bodies each consist of a continuous chromophilic reticulum in the interstices of which young yolk droplets (probably fat) are arising. After the Kolatchev or Mann-Kopsch technique, these vitellogenic masses are black, but in the best preparations or those that have been bleached, clear areas (originally occupied by the young fat) can be seen within them. Not all of the Golgi bodies show such enlargement, however, Golgi vesicles and granules still being present in the cell.

These compound Golgi bodies have been observed in teased, vitally stained material (fig. 3, horizontal arrow) discharging fat droplets and this is undoubtedly the purpose they serve in the intact oöcytes. That such elaborate inclusions in the vitally stained cells are not newly formed dye droplets is apparent from the fact that they occur in almost exactly the same form in osmic preparations that have never been subjected to vital dye treatment (compare figs. 2 and 3).

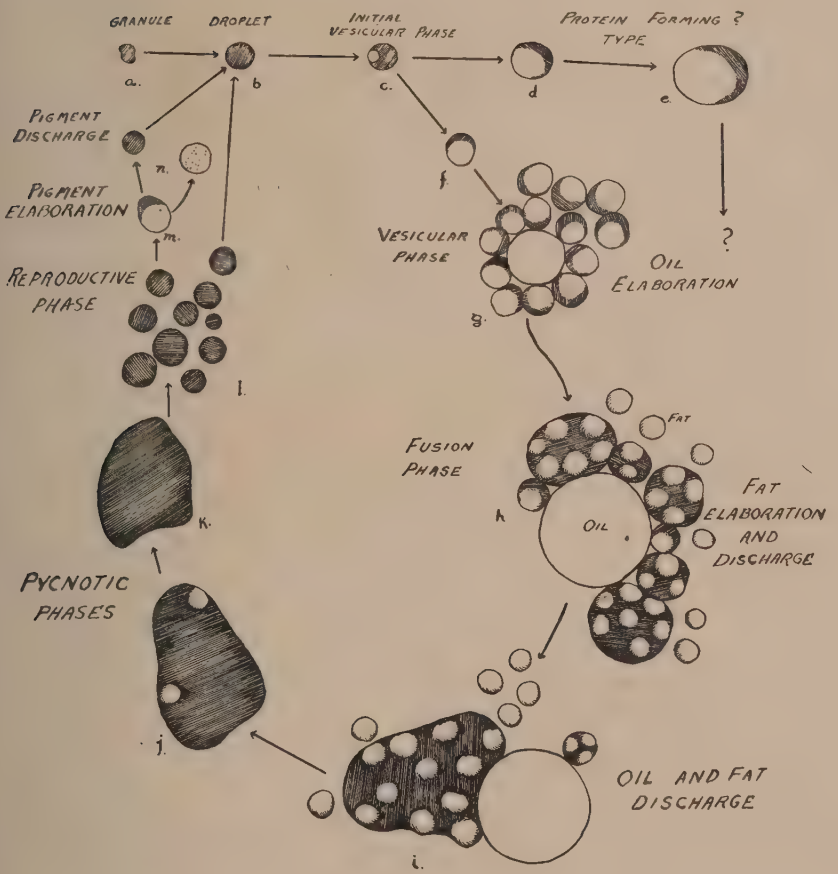

Fig. 1 Diagram, illustrating the cycle of the Golgi apparatus during the first 72 hours of development in Mytilus. Golgi granules (a) of the primary oöcyte, enlarge into droplets (b) which transform into simple vesicular forms (c) during early cleavage. A few of these, through further enlargement (d), develop on the third day into what are believed to be protein discharging elements (e). Other simple vesicular forms (f), while undergoing some enlargement, become clustered, at about 10 hours of development, and small oil (or fat) droplets appear in the center of each cluster (g). As these oily droplets enlarge, the Golgi bodies through the formation of new internal vesicles become fat forming (h) and discharge fat droplets into the cytoplasm (22 hours). By the end of the first day, fusion of the Golgi bodies takes place (h and i) followed by pycnosis between 26 and 30 hours (j and k). During the second day, the pycnotic forms break up into droplets of varying sizes (l), some of which look exactly like the original droplets (b). Others develop into simple vesicular carotenoid pigment concentrating forms (m) which, after pigment discharge (n), resemble the original droplet forms (b).

The fate of the compound Golgi bodies has not been determined, but it is believed that in the late stages of oögenesis these hypertrophied forms undergo pycnosis and break up into granules or droplets, the condition in which the apparatus is found at ovulation. This would account for the clumping that is apparent in the freshly spawned egg, the granules and vesicles not yet having had time to become scattered.

MATURATION AND CLEAVAGE

The egg, at shedding, is at the metaphase of the first maturation division (figs. 4 and 5), in which condition it awaits sperm entrance. The first polar body, at 20°C., appears in the polar depression within 10 minutes following insemination (fig. 6), while the second is formed about 30 minutes later. Maturation, in Mytilus is not accompanied by any marked rearrangement of the parts of the egg (compare figs. 5 and 7).

During maturation and early cleavage, many of the Golgi granules enlarge to form small Golgi elements of the simple vesicular type. Otherwise, they are inactive and the small vesicular types are, therefore, considered "resting" forms. When a Golgi vesicle is being stained with methylene blue, the chromophilic "crescent" is the first to show the stain because it is the thickest portion of the chromophile, but as the staining becomes more intense, the thin cortex or pellicle surrounding the vesicle becomes visible. Since the two parts of the Golgi body do not become visible simultaneously, it sometimes looks as though the vesicle were a new formation which might be interpreted as due to the presence of the vital dye. However, since the vesicular forms can be demonstrated in any good osmic preparation, there is no reason to regard them as artifacts. The appearance, in sequence, of the "crescent" followed by the vesicle, might also be interpreted as an enlargement of the Golgi body, but this too would be an error. Granules developing into simple vesicular forms first enlarge to form homogeneous droplets and then, through the enlargement of a tiny, pale, eccentric area within the chromophile, the latter becomes crescent-shaped in appearance by virtue of the pale center. Thus, the "crescent" cannot be seen as such until the vesicle is also visible (fig. 1, b–d).

During cleavage, the Golgi bodies are passively distributed to the daughter cells without any evident increase in number. No indication of any form of "dictyokinesis" has ever been observed. The mitochondria are also morphologically inactive. The great majority of them are lined up perpendicular to the egg surface, a position that many of them have occupied since the early oöcyte and in which position they

remain as the egg undergoes its changes in shape in the formation of the polar bodies (figs. 5 and 6, arrows), polar lobe formation and cleavage (fig. 7, arrows). They are completely unassociated with the polar and cleavage spindles and, like the Golgi bodies, are not thought to increase in number during cleavage. The significance of their cortical localization is not known, but since they have sometimes been thought to be concerned in cell respiration, it is possible that they may expedite the gaseous exchange at the cell surface. The mitochondria maintain their peripheral position throughout cleavage and gastrulation and thus come to lie adjacent to the lumen of the early archenteron. They have not been followed beyond the early trochophore.

YOLK FORMATION IN THE EMBRYO

During late gastrulation (about 10 hours at 20°C.) new yolk spheres, which are destined to become much larger than any in the primary oöcyte, begin to appear (fig. 9; note their absence in the early gastrula, fig. 8). When first seen, these are small translucent droplets, each in contact with one or more small Golgi bodies. In typical cases, the droplet may first appear in the center of an aggregated clump of Golgi elements (fig. 1, g). Since this development progresses from the animal toward the vegetal pole, by 15 hours all of the steps in this elaboration can be observed in a single embryo (fig. 9, A–D). The Golgi bodies maintain a close association with these new spheres until the latter reach their full size, 3–6μ in diameter (fig. 10), and it is probable, therefore, that the Golgi elements are intimately concerned in this elaboration. Such a relationship could, of course, be accounted for by supposing that as each yolk sphere enlarges it comes into contact with more and more Golgi bodies thus establishing a spatial association without physiological significance. However, the tiny yolk droplets of this type are usually completely surrounded by Golgi bodies while the largest spheres typically are not. It is also possible that these spheres are formed by a mere coalescence of the small fat granules, but there are several reasons why such an interpretation is not indicated. The fat droplets are pigmented, while the new spheres are clear and colorless. The fat granules give the impression of being rather solid particles, while the large spheres are so absolutely spherical that it is assumed that they are of relatively low viscosity. Furthermore, these spheres arise within clumps of Golgi bodies, not within groups of fat droplets. Much more plausible is the idea that through a chemical process, apparently involving the Golgi bodies, the small fat granules

serve as the primary source of the raw materials involved in the formation of the large spheres.

When these large spheres were first observed, it was thought that they might be spheres of albuminous yolk since they are about the same size and shape as the larger albuminous yolk spheres of other mollusc eggs. However, the various microchemical tests revealed them to be largely or entirely lipoidal in character. In Kolatchev preparations they are black and can readily be dissolved out with very dilute hydrogen peroxide. They are, however, clearly differentiable from the other fatty spherules which are so much smaller. Figure 11 shows both the small fat granules (horizontal arrow) and these large oily spheres (vertical arrows).

The elaboration of these large spheres is rather remarkable because this elaboration is extrasomal, i.e., outside the Golgi bodies, and each sphere appears to be formed through the mutual collaboration of many Golgi elements. During oögenesis, it will be remembered, fat formation was intrasomal, i.e., within the Golgi bodies. In the formation of the large, oily spheres we have a situation where many small Golgi bodies behave collectively as one large unit. Such elaboration results in a type of product not known in the early development of tectibranchs where, likewise, extrasomal action is not believed to take place. The absence of these spheres in the early gastrula and their invariable appearance during late gastrulation, prove beyond a doubt the ability of the developing embryo to elaborate yolk inclusions. They are very conspicuous in the unstained, living eggs.

Following gastrulation and during the transformation into the early trochophore (i.e., during the period 20–25 hours after fertilization, at 20°C.) many of the Golgi bodies show moderate hypertrophy. New vesicles make their appearance within the chromophiles — two, three or four within each (fig. 11), especially in the area of the embryo that will become the prototroch. The contents of these vesicles are discharged into the cytoplasm as solid fat spherules. Some of these new products are more deeply pigmented than the original fat droplets and can, therefore, often be distinguished from them both before and after discharge. There are now present, many of the original fat droplets which were elaborated through intrasomal action during oögenesis, newly formed fat granules of similar size and which have been similarly elaborated in the early trochophore, and the large fatty or oily yolk spheres which have been and are still being formed through extrasomal action. Undoubtedly, there has been a considerable diminution

in the number of the original fat droplets since they are probably serving as an important source of energy during development.

During the early hours of the second day of development, many of the Golgi bodies which are aggregated in clumps at the sides of the developing large oily spheres, undergo fusion (fig. 12, arrows, and fig. 1, h). This eventually results in the formation of numerous large puddingstone-like masses similar to those found in the half grown oöcyte (fig. 13). As a consequence of this fusion, there is a great decrease in the total number of Golgi elements, but there is certainly no decrease in their total volume. Clear pigmented areas (vesicles) can be seen within each of these large forms and new ones make their appearance.

These large, vitellogenic bodies can easily be seen in the unstained egg because they, as well as their products, are markedly pigmented. The Golgi elements are not noticeably pigmented in the cleavage stages, so they must acquire the pigment during development. Likewise, during the first 24 hours of development the general cytoplasm becomes colorless, so it must be concluded that the Golgi bodies have assimilated the cytoplasmic pigment during this period and have also absorbed that pigment residue remaining after oxidation of the slightly pigmented original fat droplets. The Golgi bodies discharge the pigment in a progressively more concentrated form into the new fat droplets that are continually being elaborated. The source of this new fat would seem to be the previously referred to ultramicroscopic fat droplets of the primary oöcyte. This accounts for the gradual loss of pigment from the general cytoplasm as well as for the considerable increase in size of the Golgi elements. It appears probable, therefore, that one of the functions of the Golgi bodies may be the collection and fusion of ultramicroscopical fat particles and not necessarily the synthesis of new fat.

These Golgi masses apparently have been pictured but unlabeled by Field ('22) in his brief treatment of the development of Mytilus. Stained with methylene blue, they stand out very conspicuously (fig. 13 and fig. 1, i), but they are the same size and form as in the unstained embryos. In Mann-Kopsch and Kolatchev material, treated with 2% osmic acid for 5 days, these Golgi bodies are often solid black and resemble the blackened large, oily yolk spheres, but many of them are considerably longer than wide, not spherical. Prolonged bleaching reveals the pale internal vesicles (fig. 14, arrow) and the fact that bleaching discolors the chromophobes indicates the fatty nature of the latter. These elaborate Golgi bodies are certainly larger and of

different shape than the Golgi elements of invertebrates are usually thought of as being, but these are the structures that blacken on prolonged osmication and with bleaching remain blackened longer than the fatty yolk spheres. Furthermore, in the living egg they can be seen to be formed by fusion of the small unquestionable Golgi elements. It is significant that these bodies revealed by methylene blue staining have exactly the same form and size as the blackened Golgi elements of osmic preparations of similar stages (compare figs. 14 and 15, arrows).

The fact that this hypertrophied apparatus is relatively unaltered in osmic preparations, is indicative of its more or less solid or gel-like character at this stage. There is much evidence that the Golgi apparatus shows considerable variation in viscosity from stage to stage. This seems to be in harmony with the results of others and may explain some of the discrepancy in conclusions regarding Golgi apparatus viscosity.

By the middle of the second day, fat formation has subsided and this coincides with a loss of internal structure in the apparatus and the assumption of an irregular shape (fig. 1, j and k and fig. 15). A breaking up of these pycnotic forms into droplets ensues (fig. 1, l and fig. 16), the condition found at about 48 hours. The number of droplets that may emerge in this process is very variable, there being considerable variation in the volume of the pycnotic masses and there being little uniformity in the size of the daughter droplets. This reproductive process approximately restores the original number of Golgi bodies, but is not thought to increase that number. This is the only time during the first 3 days in the development in Mytilus when the Golgi bodies are known to divide.

During the period from about 44 to 72 hours, three different processes involving the Golgi bodies take place. (1) Some formation of large fat or oil spheres through extrasomal activity seems to be occurring, but this process is very definitely on the decline and has largely ceased by 72 hours. Many of these spheres apparently are undergoing a decrease in size (fig. 16). In this process the Golgi system is not involved but instead it seems to result through a "dissolving" action of the general cytoplasm.

(2) Many of the newly formed Golgi droplets, but not others, develop into simple vesicular forms. Some of these, in the course of 24 hours, attain a rather large (4μ) size (fig. 19, horizontal arrows, and fig. 1 d and e) and look almost exactly like similar forms seen in the Mytilus oöcyte and in gastropods where they were found to be protein elaborat-

ing. Because of the small number of this type of Golgi body, it was found impossible to observe their fate, but their function is believed to be the elaboration of protein in Mytilus as well as in the gastropods. Golgi bodies of this type cannot be located on the fourth day so it is possible that the product is discharged in the meantime.

(3) Many other simple vesicular Golgi bodies that develop from the small droplet forms show only slight enlargement during the third day and the vesicles, instead of being yellow as during the early part of the second day, are very dark orange or brown (fig. 1, m and fig. 18, arrows). There is very little doubt but that these vesicles contain developing fat, but it is also apparent that a considerable quantity of carotenoid pigment is lodged in the fatty medium. Each deeply pigmented fat droplet is discharged as the chromophile again assumes the droplet form (fig. 1, n). During the third day, typically only one pigmented fat droplet is formed within each Golgi body and the latter remains small and relatively simple. In these cases, the chromophobe is a droplet of fat containing pigment. This phenomenon probably amounts merely to a concentration of pigment already present in the egg, since few if any animals are able to synthesize carotenoids. Since such pigments are usually found dissolved in fat, and since, as we have seen, all of the fat in the Mytilus embryo arises under Golgi body influence, it is to be expected that the pigment would of necessity appear in association with the Golgi elements.

Fat, probably some protein yolk, and carotenoid pigment make their appearance or reappearance in Mytilus in association with the Golgi system, but these are the only contents of the embryo with which the Golgi bodies appear to be concerned during the first few days of development. No relationship could be detected between the apparatus and the young organic shell (fig. 17) which appears on the second day and is very conspicouous by 48 hours. In fact, there has been nothing in these observations to indicate that the Golgi system possesses any direct morphogenetic ability.

DISCUSSION

Several factors have made the study of Golgi body activity in Mytilus particularly difficult. One of these is the small size of the egg and another is the presence, after the middle of the second day, of the young shell which soon covers most of the embryo and obscures the events taking place within. As a result, the observations recorded here are not quite so clear cut as they were in the previous study of the nudibranchs and tectibranchs, but there seems to be no way at

present of obviating this difficulty. Nevertheless, these observations do provide additional information on the activity and function of the Golgi apparatus in developing molluscs and although, in general, these observations confirm the earlier work, certain contrasts between the pelecypod Mytilus, and the tectibranch Navanax, can be recognized:

1. In Navanax, fat and protein elaboration appear to be of about equal importance, fat formation being very conspicuous in the trochophore and protein discharge characterizing the veliger. In Mytilus, on the other hand, fat elaboration greatly predominates during the larval stage and it is of interest to note that the veliger, the protein-forming stage in the tectibranch, is absent in the pelecypod.

2. Large oily yolk spheres are elaborated in Mytilus, but not in Navanax. After these spheres in Mytilus were found to be largely lipoidal in character, the somewhat similar large yolk spheres in Navanax were retested, but they were found to be, at least largely, of a protein nature. It is to be noted, further, that these spheres in Mytilus arise through extrasomal Golgi body action, a type of behavior not encountered in Navanax.

3. In Mytilus, a fusion of Golgi elements precedes the later stages of fat elaboration and pycnosis while in Navanax, no such fusion was observed. This discrepancy in behavior may be correlated with the need of the embryo for new Golgi elements. In Navanax, the breaking up into droplets clearly increases the number of Golgi bodies, while in Mytilus there appears to be no increase during the stages studied and the embryo, likewise, is not increasing in size. In both Mytilus and Navanax, however, division follows pycnosis. The size of these daughter Golgi bodies varies considerably in Mytilus, while in Navanax it is relatively constant.

4. Golgi bodies in Mytilus are incidentally concerned in the concentration of carotenoid pigment. No such activity in the early stages of Navanax has been observed.

Crescent-shaped inclusions (probably vesicular Golgi bodies) were observed in the cytoplasm of fixed eggs long before the almost universal distribution of Golgi bodies in animal cells was realized. Lillie as long ago as 1906, noting these in the Chaetopterus egg, suggested that they were partly digested yolk spheres. Actually, however, in Mytilus these apparent spaces in the spheres are at first fluid and later more or less solid vesicles which are eventually discharged as yolk. A feature of early development that has been repeatedly overlooked is that yolk formation may transpire during embryonic develop-

ment as well as during oögenesis. As a matter of fact, the observations reported here offer some evidence which suggests that some of the yolk formed during oögenesis is also consumed during that period as well as during the post-fertilization stages.

Payne ('32) figures vesicular osmiophilic inclusions ("gray bodies") from oöcytes of the beetle Cicindella (fig. 52, p. 581) which closely resemble the compound vesicular Golgi bodies of Mytilus. Although "possible transitional stages between Golgi bodies and 'gray bodies'" were observed in the bug Gelastocoris, Payne preferred the view that the latter were not Golgi inclusions and arose "de novo". Inclusions similar to the compound Golgi bodies of the oöcyte and larva of Mytilus were also noted by Payne in the osmicated eggs of the stonefly (p. 575), but they were not identified as Golgi bodies. It seems to me that so much emphasis has been placed on the fact that many osmiophilic cytoplasmic inclusions are not to be called Golgi bodies, that we may frequently have been over cautious in this regard and as a consequence have overlooked some of the most interesting examples of Golgi body activity. Certainly, due caution should be exercised in identifying the Golgi substance, but it is just as erroneous to overlook Golgi inclusions as it is to identify as Golgi material elements that are not.

The form and activity shown by the Golgi bodies in the eggs of molluscs, give some support to certain of the theories advanced by Hirsch ('39) which were the result primarily of that investigator's studies of vertebrate gland cells. Hirsch found that the vesicular Golgi bodies (Golgi systems of that author) arose from small, chromophilic granules (granule B_1). Such vesicles were found to consist of a chromophilic cortex (Golgi externum) and a chromophobic center (Golgi internum). Hirsch also recognized compound vesicular forms (polysystem) and considered the chromophobe to be the forerunner of the Golgi body product. Since there are cases, as exemplified in the elaboration of the large oily yolk spheres in Mytilus, where the product arises outside the Golgi body, the term "internum" for the developing product is not always appropriate. The terms "chromophile" (or "osmiophile") and "chromophobe" (or "osmiophobe") are, therefore, believed to be preferable, since the chromophobe is always the product whether elaboration is intrasomal or extrasomal. It is a matter of some significance, however, that Hirsch and I have arrived independently at such similar conclusions regarding the structure and function of the Golgi apparatus as the result of study based primarily

on such widely different zoölogical materials as the vertebrate gland cell and the invertebrate egg.

In the introduction it was mentioned that there has been a considerable difference of opinion regarding the origin of yolk. Some of this difference of opinion may be due to the fact that all animals do not behave similarly in this regard, but it is very certain that some of the discrepancy is due to different interpretations of the same process. In the centipede Lithobius, for example, Harvey ('29) points out that four different authors, writing in 1924 and 1925, reached four different conclusions on the matter of vitellogenesis in that one form. At least three of these investigators must have been in part or wholly in error in their interpretation. This being the case, it seems very probable that errors have been made in the interpretation of yolk formation in other species. Some of the reasons for this are made clear in Mytilus and Navanax. In these forms, especially during oögenesis and the early cleavage stages, many of the Golgi bodies are in the form of homogeneous granules which, in fixed preparations, stain exactly like mitochondria, like the chromophobic components of the Golgi vesicles and like small protein yolk spheres or plates. Furthermore, since these various elements also resemble each other in size and shape, they are subject to misidentification. Probably the Golgi granules have frequently been identified as granular mitochondria. An observer, noting the enlargement of these granules (Golgi granules) into fuchsinophilic spheres (Golgi vesicles), might readily conclude that the mitochondria gave rise to the fuchsinophilic albuminous yolk. Another investigator, noting chromophilic or osiomophilic rims "attached to" such spheres could reasonably conclude that the albuminous yolk was formed by the Golgi bodies, or that "lipoidal matter" was "contributed by the Golgi bodies to swollen, yolky mitochondria". It is not certain that such errors have been made, but it seems appropriate to point out these possibilities. Conclusions pertaining to vitellogenesis based solely on the observation of fixed material must be considered questionable. Certainly, in Navanax, both embryonic fat and protein yolk and, in Mytilus, fatty yolk and probably protein yolk, as well as large oil drops, arise in intimate association with the Golgi apparatus. This does not, of course, preclude the possibility of other origins in other forms or even in these species. It does not, however, seem appropriate to assume, as many have done, that because the apparatus is involved in the elaboration of fat or lipids, it cannot also play an important part in the elaboration of protein. In vertebrate sero-zymogenic cells, as is well known, the apparatus is in all probability concerned with protein

formation. The question of why albuminous yolk is traced to the Golgi apparatus by some authors and to the nucleolar extrusions by others, can be answered only after further study.

SUMMARY

1. The Golgi system has been followed continuously in the living cells during the first 3 days of development in Mytilus. In the primary oöcyte, the apparatus consists of chromophilic granules and small chromophilic droplets and vesicles. The latter each consist of chromophilic cortex and chromophobic center. The cortex is usually thickened on one surface, forming a bowl-shaped tubercle which, in fixed preparations, appears crescent-shaped, or sometimes rod-shaped. Many of the Golgi granules ("reserve" granules) enlarge to become simple vesicular Golgi bodies ("resting" or inactive forms) during maturation and cleavage.

2. During late gastrulation and in the early trochophore, numerous Golgi vesicles aggregate and in the center of each clump a hyaline oil drop develops. These become free of the Golgi bodies on attaining a diameter of from 3–6μ. They are apparently elaborated through "extrasomal" Golgi body action.

3. In the very early trochophore, both aggregated and unaggregated Golgi bodies enlarge and new internal vesicles appear which apparently are soon discharged as yolk granules about 1μ in diameter. These are presumed to be fat droplets since they contain considerable carotenoid pigment and are the same size as fat granules already present. These yolk droplets are elaborated through "intrasomal" Golgi body action.

4. Enlargement of the Golgi bodies is soon followed by fusion, resulting in large, puddingstone-like masses, which continue to discharge 1μ fat droplets. Each mass consists of a continuous chromophilic or osmiophilic component and a dispersed chromophobic component (the vesicles).

5. Pycnosis of each vitellogenic mass takes place as the yolk-forming period subsides and each mass becomes resolved into droplets of different sizes which become scattered. Many of these droplets soon exhibit pale, vesicular centers which, however, appear light brown and apparently contain pigment in considerable concentration, associated with fat. These vesicles are discharged as fatty pigment-containing spherules during the second and third day of development.

6. As the lightly pigmented fat droplets of the primary oöcyte are gradually consumed during development, the Golgi bodies apparently

have the ability to collect, store and concentrate the pigment and discharge it later in more and more concentrated form. This absorbing power also seems to involve pigmented ultramiscropic fat particles of the fertilized egg. This diffusely distributed pigment of the single-celled stage gradually disappears as the Golgi elements become more deeply pigmented during the first day of development.

7. A small number of Golgi bodies appear to engage in protein elaboration during the third day of development and during oögenesis but, in general, protein elaboration is a relatively inconspicuous process. Any protein yolk formed during oögenesis disappears from the egg by the time of laying and hence is probably consumed during the later stages of gametogenesis.

8. The apparatus does not participate in the formation of the young organic shell, nor does it appear to possess any direct morphogenetic power. It is not directly concerned in the utilization of the formed yolk inclusions.

9. The primary oöcyte is believed to be devoid of formed protein yolk, but is loaded with fat droplets elaborated by the Golgi apparatus during oögenesis, through the formation of puddingstone-like masses in the oöcyte. These apparently subsequently break up into granules and vesicles, the condition in which the apparatus is found at ovulation.

10. Fusion of the Golgi elements is a conspicuous process in the larva and compensates for the multiplication of Golgi bodies that occurs following the pycnotic phase. Fusion also probably occurs during oögenesis.

11. All of the positively identified mitochondria in Mytilus are rod-shaped or short filaments, although these may arise from granules in the early oöcytes. Mitochondria early become localized in the egg cortex and remain inactive during maturation, polar lobe formation and cleavage. Mitochondria and Golgi bodies are both passively distributed during the cleavages and show no evidence of multiplication.

12. There is evidence that, in keeping with its variations in form, the Golgi apparatus shows considerable variation in viscosity, being more or less fluid as a simple vesicle, but semi-solid or gel-like in the puddingstone-like condition.

13. The observations reported here support in some details the views of Hirsch ('39) regarding the structure and function of the Golgi apparatus.

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PLATE 1

EXPLANATION OF FIGURES

All stated magnifications are approximate.

2 Mid-ovarian oöcyte of Mytilus (× 1000) showing Golgi inclusions — granules, simple vesicles (diagonal arrow) and compound vesicular forms (horizontal arrow). Mitochondria are not visible. Modified Flemming and iron hematoxylin. Compare with teased material supravitally stained with methylene blue, figure 3.

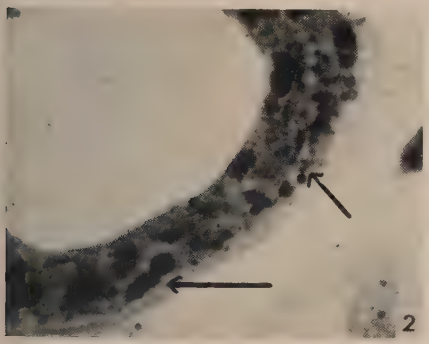
3 Golgi bodies isolated from Mytilus oöcytes after supravital methylene blue staining (× 2000). Note pale internal vesicles. Simple vesicular form, probably protein forming, at diagonal arrow; compound vesicular fat-forming type at horizontal arrow. Compare with section of fixed material, figure 2.

4 Sagittal section of unfertilized egg (× 600) showing distribution of Golgi bodies and fat spheres (not separable). Note their absence in the spindle area and egg cortex. Mann-Kopsch technique.

5 Sagittal section of unfertilized egg (× 600) showing first maturation spindle, cortical concentration of rod-like mitochondria (arrows) and abundant Golgi granules and simple vesicular forms in the endoplasm. Some clumping of the Golgi bodies is apparent. Yolk is barely visible. Regaud fixation followed by aniline acid fuchsin and methyl green.

6 Sagittal section (× 600) of egg at metaphase of the second maturation division, following sperm entrance. Note static condition of mitochondria (arrows) and Golgi bodies. Regaud fixative followed by crystal violet.

7 Sagittal section (× 600) showing first cleavage spindle, cortical concentration of mitochondria (arrows) and inactive condition of both mitochondria and Golgi bodies. Regaud fixative followed by crystal violet.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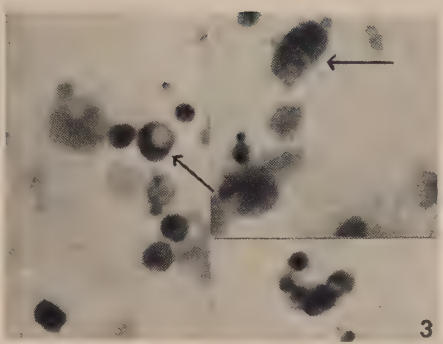
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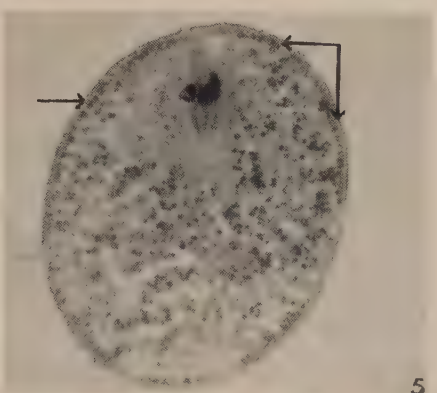
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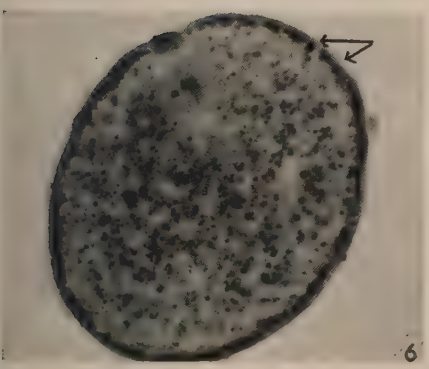
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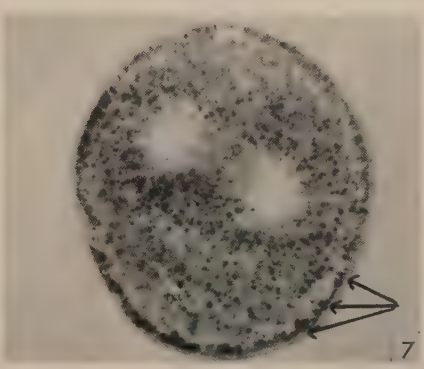
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PLATE 2

EXPLANATION OF FIGURES

8 Early gastrula (× 600) to show the absence of large oily yolk spheres of late gastrula, figure 9. Supravital methylene blue technique.

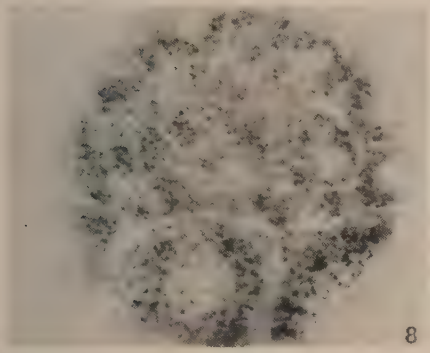
9 Mytilus embryo immediately following gastrulation (about 15 hours after fertilization) showing steps in elaboration of large oil spheres (× 1200). Typically, the Golgi bodies first become clumped (A), an oil drop appears within the clump (B) and enlarges (C). Finally, as the droplet attains full size it becomes dissociated from the Golgi bodies (D). Note that the Golgi bodies are fairly uniform in size. Supravital methylene blue technique.

10 Mytilus embryo 19 hours after fertilization (× 2000), showing intimate association between oily yolk spheres and the chromophilic Golgi elements. Supravital methylene blue technique.

11 Embryo at 22 hours (× 2000). Golgi bodies show enlargement, more variation in size and are becoming more vesicular. Extrasomal oil elaboration is still taking place, but small fat droplets arising through intrasomal action are being discharged into the cytoplasm. Vertical arrows indicate large oily spheres; horizontal arrow indicates small fat droplets for comparison. Supravital methylene blue technique.

12 Slightly older embryo (× 2000) showing early stage in Golgi body fusion (arrows). Discharge of small fat droplets continuing. Supravital methylene blue staining.

13 About 25 hours after fertilization (× 2000). Future prototroch toward lower right. Compound vesicular vitellogenic Golgi masses formed through fusion which is still in progress. Formation of large spheres subsiding. Supravital methylene blue technique.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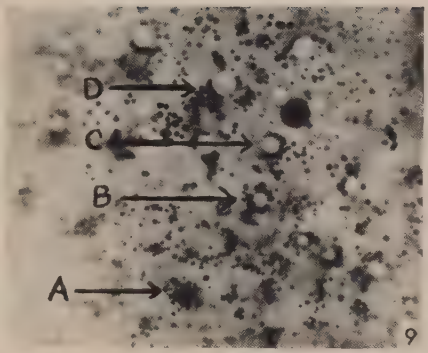

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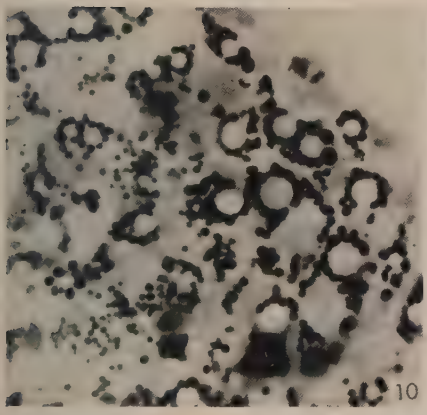
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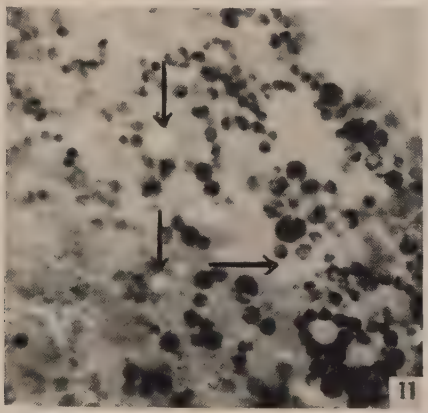
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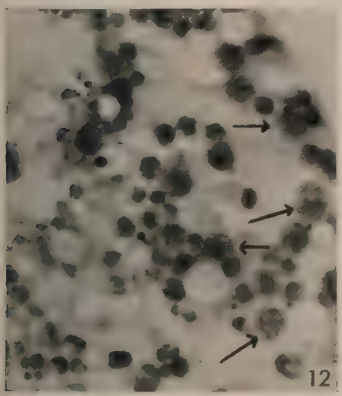
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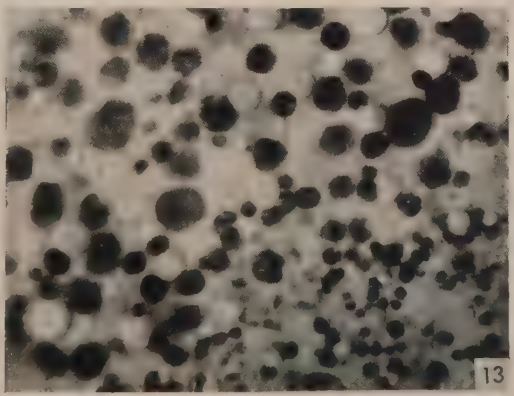
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Contribution No. 223

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LIAM EMERSON RITTER: NATURALIST AND PHILOSOPHER[1]

OFESSOR RITTER had entered his eighty-eighth at the time of his death a few months ago (Janu-0). His advanced age must have come as a sur- to many, for as recently as 1938 he had brought n impressive volume of biological fact and inter- tion, and he had contributed to some of our sci- c journals even later than that. Ritter got a what delayed start in his scientific career, but he than compensated for this by exceptional pro- vity at an age when most of us lapse into silence. lliam Emerson Ritter was born in Hampden, Wis- n, on November 19, 1856. Much of his boyhood spent on a farm, and to this period of his life as fond of referring later. It was here, no t, that his life-long love of nature commenced. raduated from the State Normal School at Osh- in 1884, and engaged in public school teaching in ative state before carrying his education to higher s. In 1888 he received his B.S. from the Univer- of California, and his doctorate was attained at ard in 1893, when he was 36 years old. In June, , he married Dr. Mary E. Bennett, whom he char- izes in a dedication as "my severest critic and helper." There is no doubt of the important part h she played in various phases of his career. Rit- quest of knowledge and adventure took him to les and Berlin in 1894–95; later to England, n and various points in the Pacific area. He a member of the carefully selected party of nat- sts which comprised the Harriman Alaska Ex- tion of 1899. His academic positions ranged that of instructor in biology at the Univer- of California to the headship of the department ology, in the same university, a position which he until he assumed directorship of the Scripps In- tion of Biological Research in 1909. He retained ofessorship in the department, however, until he retired as "professor emeritus" in 1923. Ten s later the degree of LL.D. was bestowed both Professor Ritter and upon Dr. Mary Bennett er, his wife.

tter's first published paper of which I find record t with "The parietal eye of some lizards from the ern United States,"[2] thus appearing in his thirty- year. Two years later came his doctor's thesis, the Eyes, the Integumentary Sense Papillae, and the Integument of the San Diego Blind Fish (*Typhlogobius californiensis Steindachner*)."[3]

Up to nearly 1910, Ritter's biological contributions lay predominantly in the fields of taxonomy and morphology, his special groups being the tunicata and enteropneusta. The texts and figures of these papers (the figures mainly of his own execution, it would seem) bear witness to a high degree of care and thoroughness in his observation and delineation. But questions of interpretation frequently occupied his attention, as is evidenced, for example, by such a title as "*Halocynthia johnsoni* n. sp., a Comprehensive Inquiry as to the Extent of Law and Order that Prevails in a Single Animal Species."[4]

These morphological and taxonomic studies gave place rather early to behavioristic and philosophical ones, and it is probably these last by which Ritter is more largely known. His stature as a biological philosopher can probably be fairly measured only by another philosopher. His writings reveal extensive acquaintance both with the literature of philosophy and of the history of science, such an acquaintance as I believe few living zoologists possess. And they reveal preoccupation with certain philosophical problems throughout much of his life. His association with Howison at the University of California appears to have been partly responsible for this trend, though the viewpoints of the two men differed very widely. Ritter was primarily a naturalist, Howison a subjective idealist.

Ritter's outstanding published work in the field of biological philosophy bears the title "The Unity of the Organism, or the Organismal Conception of Life."[5] This two-volume book has as its "central idea" that "The organism in its totality is as essential to an explanation of its elements as its elements are to an explanation of the organism." The contrary viewpoint he characterizes as the "elementalist" one, and this viewpoint he seems to attribute, in varying measure, to all other living biologists.

The metaphysical implications of Ritter's "central idea" are matters which the present writer is quite unqualified to discuss. That his position is a "vitalistic" one Ritter denies emphatically, and his denial carries conviction. Superficially, at least, there seems to be much in common between his views and ones set forth during several past decades under such names as

Contributions of the Scripps Institution of Oceanogy, New Series No. 223.

Bull. Mus. Comp. Zool., Harvard, January, 1891.

[3] *Ibid.*, April, 1893.

[4] University of California Publications in Zoology, November 15, 1909.

[5] Richard G. Badger, 1919.

"emergent evolution," "creative evolution," "creative synthesis" and "holism." He insists upon an all-out physico-chemical basis for vital phenomena, but he equally insists upon the appearance of entirely new attributes only potentially present in the chemical elements.

Ritter challenges the mechanistic physiologist to state just what he means by expressing a vital phenomenon (say a dog's scratching reflex) in "terms" of the physico-chemical elements involved. This "can be done in one and only one way, namely, by adding to the attributes, that is to the 'terms' which inorganic chemistry recognizes in the chemical agents concerned, just those attributes and terms which the dog's scratch reflex requires in order that the elements may explain the reflex." For brevity's sake, he coins the whimsical abbreviation "doscarex" for the reflex in question, and proceeds to discuss the "doscarecious powers of the oxygen, carbon and so forth," so unforeseeable by the mere chemist![6] This is rare humor. Is it not possibly sound philosophy?

In view of these forthright assertions regarding the relations of the organic to the inorganic, it is rather surprising to find Ritter aligning himself with those few biologists who seriously question the ultimate derivation of the former from the latter, and take refuge in such conceptions as the "panspermia" hypothesis of Arrhenius and others. He could scarcely have questioned the force of such contrary arguments as have recently been set forth by Oparin,[7] who removes one of the difficulties in the way of accepting the earlier chemical origin of living matter by making it plain why this process could hardly be expected to occur under present-day conditions.

"The Unity of the Organism" was surely a valuable contribution to the literature of theoretical biology, and may still be read with profit twenty-five years after its publication. It is packed with important material, interestingly presented, and much of the discussion is still stimulating. Ritter, I think, was disposed to magnify the differences between his own points of view and those held by other biologists whom he characterized as "elementalists," a circumstance which gave to some of his utterances a needlessly disputatious tone. This was accentuated by his failure, at times, to discern the meaning behind the esoteric sounding terminology of the rather bumptious young science of genetics. Again, Ritter's own utterances did not always lack a cabalistic tinge, as when he asserts his belief that "every living individual organism has the value, chemically speaking, of an elementary chemical substance"; and he gives no biochemical grounds for the hypothesis that all manifestations of life, including the psychical ones, "result fro chemical reaction between the organism an respiratory gases"—oxygen being almost certain effective one. Even the format was against the s of "The Unity of the Organism." One does not narily look beneath such small covers for a work considerable importance.

Dr. Ritter's last work of book dimensions certainly one of his best, was "The California V pecker and I," to which we must add the subti characteristic of the author, "A Study in Compa Zoology, in which are set forth numerous fact reflections by one of us about both of us."[8] Th ume as a whole is as unique as the title, comprisin tions designated "Chiefly Woodpeckers," "Chiefly of Us," "Mind, Brain, Conduct," and "Chiefly self." Here we are taken in review through R own more than twenty years' observations on the a storing and other instincts of this remarkable followed by excursions into anatomy, physiology, logeny, psychology, sociology, epistemology and e The reader may fail to detect any great degr coherence at all points in the narrative, but t not necessary to the enjoyment of it.

No account of Professor Ritter's career would all complete without considerable mention o Scripps Institution at La Jolla, which may, to tain extent, be regarded as his monument. The Sc Institution for Biological Research was the ou of a series of endeavors by Dr. Ritter and some o to create a seaside laboratory in connection wit department of zoology at the University of Calif These earlier ventures, commencing as far ba 1892, included the establishment of very temp quarters successively at Pacific Grove, Santa Ca Island, San Pedro, Coronado and within the v of La Jolla. Building upon the present ground commenced in 1909, and two years later the then Diego Marine Biological Association" deeded property to the University of California, unde title "Scripps Institution for Biological Resea Omission of the word "marine" was deliberate.

It was during this period of unrestricted biol outlook that the present writer became a member o institution's staff, and for many years thereafte studies of the races of wild mice were regarde altogether relevant to the liberal, if somewhat r lous, "program" of the "Biological Station." In generous funds were allotted to them throughout. Scripps's own avowed interests were in human p lems, and in biological problems only in so far as might have human implications. At one time h came mildly interested in a project to establi colony of anthropoid apes upon our campus,

[6] "Unity of the Organism," Vol. 2, p. 204.

[7] "The Origin of Life" (Trans. by S. Morgulis), Macmillan, 1938.

[8] University of California Press, 1938.

hat later even a department of sociology. But r enterprise got beyond the talking stage. How- Scripps's own support of the institution was rily in behalf of Ritter. He more than once hat what he and his sister were really doing endowing Ritter," but this was probably a bit ippsian hyperbole.

already intimated, the first plan of Ritter and sociates had in view the building up of a marine ical station. Writing as late as 1911, he stated ogram as being, in essentials, "a biological survey waters of the Pacific adjacent to the coast of ern California." Rather a stupendous project will say who have had anything to do with ical surveys! Later, as stated, the word marine ropped, and mice and monkeys were regarded rmane to our "program." But the next tack in stitution's somewhat variable course took it well ward again. The word "oceanographic" was in- rated into the official name of our station, and the irector, succeeding Dr. Ritter upon his retire- was selected with particular view to his qualifica- in this field.

motives behind this change on Ritter's part are hat inscrutable. Its wisdom, at the present date, urely academic question. The Scripps Institu- f Oceanography is very much a going concern, nder wartime conditions. Most fortunately the "oceanography" has been interpreted very ly by both of Ritter's successors, and biological work, though changed somewhat in scope, has been liberally supported.

Dr. Ritter's cooperation with E. W. Scripps and others in the founding of the well-known scientific news agency, Science Service, can hardly be discussed here, though it is important to note that it was to Ritter to whom Scripps intrusted the task of interesting and organizing the scientific supporters of the enterprise.

To all who really knew him William E. Ritter looms large, not only as a most lovable personality but as a scientist of much originality and forcefulness. His constant interest, both in the scientific achievements and the personal welfare of the members of his staff at the Scripps Institution, was an outstanding feature of our lives throughout his term as director. Every new bit of information gathered by one of us, whatever its subject-matter, seemed to fit in some way into his framework of thought, and often as not served to illustrate some one of his favorite ideas. He was fond of discoursing with us, singly or in groups. These discourses were often stimulating. But we did not always find them easy to follow. Former President Wheeler, of the University of California, once characterized Ritter, so I am told, as "a great soul struggling for utterance." But this epigrammatic description hardly does him justice, for these "struggles" were not infrequently productive of impressive results.

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SEXUAL DIFFERENTIATION IN MOLLUSKS

II. GASTROPODS, AMPHINEURANS, SCAPHOPODS, AND CEPHALOPODS

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(Contribution from the Scripps Institution of Oceanography, New Series, No. 224.)

I. GASTROPODS

N A preceding review (Coe, 1943) it was mentioned that, among the 10,000 or more species of pelecypod mollusks, there is found every grade of sexual differentiation, from those species that are strictly of separate sexes to rs that are functionally hermaphroditic. Furmore, in certain species the functional sexual se may change during the individual's lifetime. as found convenient to classify all these aspects xuality into two main groups, namely, (A) amxuality (monoecism, hermaphroditism) and unisexuality (dioecism, gonochorism). There four more or less intergrading categories of isexuality: (1) functional ambisexuality (funcal hermaphroditism), (2) consecutive sexuality, rhythmical consecutive sexuality and (4) alterve sexuality. The same categories are found he gastropods and, indeed, in some other groups nimals and plants.

f the more than 20,000 species of living gastros, approximately half are hermaphroditic when y adult, in contrast with the pelecypods, most which are of separate sexes (Coe, 1943). Two he three orders of the class Gastropoda, namely sthobranchiata and Pulmonata, are hermaphroc, with few, if any, exceptions. The third er, Prosobranchiata, contains representatives of all of the five categories of sexuality. In this order the type of sexuality is not in all cases characteristic of the family or even of the genus, for two species of the same genus may differ in their sexual conditions.

Most of the gastropods are provided with a copulatory organ, or phallus, in the male sex, in the male phase, or in the hermaphroditic condition, as the case may be, whereby the sexes or the sexual phases can be distinguished externally (Figs. 1, 3, 4, 5). There are also some examples of sexual dimorphism in shape of shell. Moreover, some of the dioecious species are characterized by differences in rate of growth and longevity of the two sexes. Males, in such cases, grow more slowly and die at an earlier age than females in the same population. Consequently the sex ratios in a group of small individuals will differ widely from a group consisting of an equal number of large individuals. The group of small ones, if they are also young, will consist of equal numbers of males and females, while a group of the largest and oldest individuals will contain only the longer surviving females (Pelseneer, 1935; Van Cleave and Chambers, 1935; Gallien and de Larambergue, 1938).

In a few families, including both unisexual and ambisexual species, the phallus is absent and the reproductive cells of both sexes are discharged into the water, as is usually the case in the pelecypods.

Ovoviviparity. In numerous species of prosobranchs and pulmonates and in a few opisthobranchs the eggs are fertilized and undergo development to the adult form in the uterine portion of the oviduct. This may occur either in ambisexual or unisexual species. In some species certain individuals may be oviparous while others of the same species, but in a different locality, may be ovoviviparous.

Parthenogenesis. This has been conclusively demonstrated in two species of pectinibranch prosobranchs. In these species, *Hydrobia* (*Paludestrina*) *jenkinsi* and *Campeloma rufum*, males are unknown, and the hereditary line is evidently continued by females exclusively (Mattox, 1937, 1938). Other examples have been reported but without satisfactory proof; most of them are evidently cases of self-fertilization and not parthenogenesis.

Neoteny. As occurs also in other groups of both animals and plants, the sexual conditions in the gastropods are sometimes complicated by precocious sexual maturity. This may lead to the production of sexual cells when the individuals are very young, sometimes before the larval development has been completed. This primary sexual phase is often more or less aborted and may be of a different type of sexuality than that in which the individual will function as an adult. In most cases of neoteny a brief male phase precedes a later h maphroditic condition.

A. AMBISEXUALITY; MONOECISM; HERMAPHRODITISM

In addition to all species of opisthobranchs a pulmonates, numerous examples of this type of s uality have been reported among the 12,000 or m species of prosobranchs. They include spec of *Acmaea, Angustispira, Bathysciadium, Cal traea, Capulus, Crepidula, Crucibulum, Cocculi Enteroxenos, Entocolax, Entoconcha, Entosiph Janthina, Marsenina, Odostomia, Onchidiopsis,*

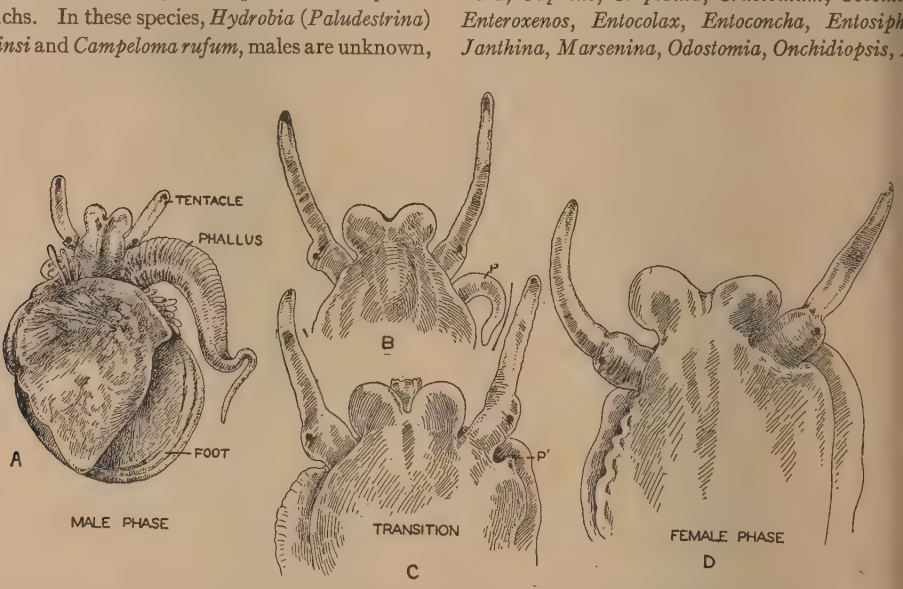

FIG. 1. CONSECUTIVE SEXUALITY IN CREPIDULA PLANA

A, dorsal surface of individual in male phase after removal from shell, showing the relatively large size of fully developed phallus. B, C, D, outlines of anterior portions of bodies during sexual transformation and female phase, showing regression of phallus (*p*). All figures drawn at the same magnification.

tella, Puncturella, Stilifer, Valvata and *Velutin* The sexuality of some of these, as will be explain in the following sections, is more strictly of t consecutive type, the rhythmical consecutive typ or the alternative type. The life histories of ma others are not sufficiently known to permit a de nite classification. As in so many other groups animals, ambisexuality is commonly associat with parasitism.

1. Functional hermaphroditism; Functional ambisexuality

In this aspect of sexuality there is no inco patibility between male and female characteristi and each normal individual is provided with

mplete set of organs of both sexual types. The nad produces both sperm and ova, and the geni- l ducts are often differentiated into male and male portions. The male accessory organs usu- y, but not invariably, consist of sperm duct, with minal vesicles for the storage of sperm, accessory cretory glands and eversible phallus. The female cessory organs are usually differentiated into iduct, uterus, seminal receptacles, and vagina, addition to accessory glands associated with pulation and with the secretion of yolk and egg ells or capsules.

In most species with this type of sexuality, all ature individuals experience the mating instincts both sexes and during copulation the sperm is ansferred reciprocally, either simultaneously or ternately, to both members of the mating pair. ross fertilization is thereby generally assured. some of the pulmonates, however, the gonad oduces ripe eggs and sperm simultaneously, and ch individuals may reproduce by self-fertiliza- on, generation after generation, when experimen- lly isolated (Crabb, 1927; Pelseneer, 1935; Ikeda, 37; de Larambergue, 1939).

Colton and Pennypacker (1938) have followed ch an isolated, self-fertilized line through 93 nerations, extending over twenty years. The gs are fertilized within the body, either in the ary or in the genital duct. Accidental self- rtilization has been reported for other groups of stropods.

At the end of the reproductive season the phallus ay sometimes become atrophied or it may be awed off by the individual, to be replaced by generation at the beginning of the following ating period.

As a general rule there is a tendency towards otandry. The sperm may be produced in the nbisexual gonad and then discharged before the a are fully mature, or a definite male phase may ecede the hermaphroditic condition. The extent protandry can sometimes be increased experi- entally by an unfavorable environment. This pe of sexuality thereby intergrades with rhythmi- l consecutive sexuality, as is more fully explained a following section.

2. *Consecutive sexuality*

In this type of sexuality there is but a single ange of sexual phase, or sex reversal. Such dividuals function as one sex when young and a different sex when fully adult. Under normal conditions the initial sexual phase is usually male, while the adult phase is either female or hermaphroditic (Figs. 2–5). The two phases may intergrade or they may be sharply separated, as in the pelecypods and many other groups of invertebrates, as well as in some plants and a few fishes.

The histological basis of this type of sexuality depends upon the presence of an essentially ambisexual gonad, associated with an hereditary mechanism which initiates the activity of one of the two types of sexual cells in early life and of the other type after the individual has already functioned in one of the two sexual phases.

Consecutive sexuality is especially characteristic of the family Capulidae (Calyptraeidae), containing 7 genera and about 150 species. The best known examples of these belong to the genera *Calyptraea*, *Crepidula*, and *Crucibulum*. All the species of these genera, of which the life histories are known, function as males when young and later change to the female phase. The female phase continues for the remainder of the individual's life (Figs. 3, 4, 5).

These mollusks lend themselves readily to experimentation, for they are sedentary when adult and thrive in screened boxes immersed in the sea, requiring no special feeding. The successive sexual phases of each individual can be followed by allowing the animal to attach itself to the inner surface of a bottle from which the bottom has been removed.

In some of these species the transformation of the sexual phase is gradual and the individual passes through a series of ambisexual stages. In others the succeeding phases are sharply separated, with a return to a condition of sexual immaturity after the termination of the male phase. The change in sexuality involves not only the gonad but also the genital ducts and copulatory organs, as well as the associated behavioristic instincts.

In the Capulidae all normal individuals reach the functional male phase when they are very young and when they are only a small fraction of their definitive size (Figs. 1–5). They are then motile. In most species, each young snail tends to creep about until it finds an individual of the same species in the female phase, whereupon it attaches itself to the dorsal side of the female's shell in a position adjacent to the female copulatory organ (Figs. 3, 8). Here it may remain for several months or until ready to transform to the female phase and, in some species, even after

reaching the female phase. Not infrequently two or more male-phase individuals attach themselves, temporarily or permanently, to the shell of a single female. In other species the males usually occupy positions in the vicinity of the female and move to the mating position at night (Coe, 1938b).

male phase the residual spermatogenic cells in the gonad become cytolyzed and absorbed, together with such sperm as may remain in the seminal vesicles. Later the seminal vesicles are gradually transformed into a portion of the future oviduct. The phallus then atrophies, leaving only a small

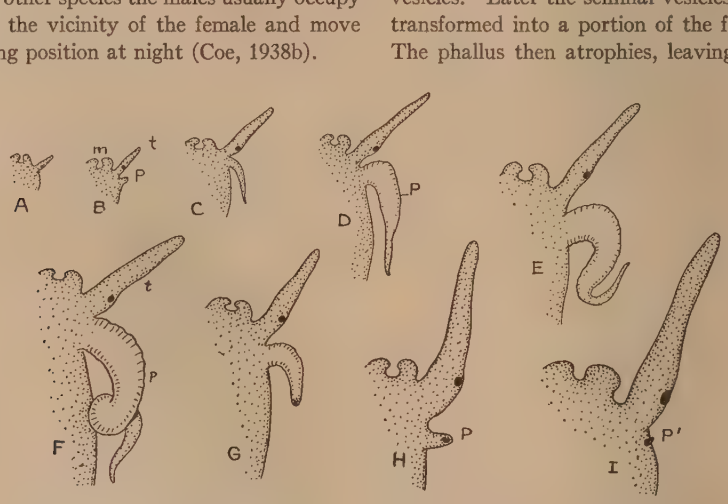

FIG. 2. CONSECUTIVE SEXUALITY IN CRUCIBULUM SPINOSUM, SHOWING SUCCESSIVE STAGES IN THE DEVELOPMENT AND ABSORPTION OF PHALLUS

A, sexually immature. B, C, D, early male phases with spermatogenesis in progress. E, F. functional male phases. G, H, stages in transition to female phase. I, female phase, with rudiment of former phallus; *m*, mouth; *p*, phallus; *t*, tentacle, with ocellus.

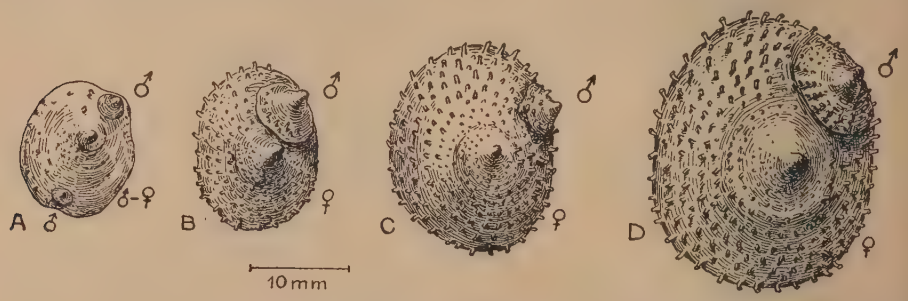

FIG. 3. CONSECUTIVE SEXUALITY IN CRUCIBULUM SPINOSUM

A, shell of individual in process of transformation from male to female phase, with two immature male-phase individuals on surface. B, C. younger and older female-phase individuals with males in mating position. D, old female, near end of life, with mated male-phase individual nearly ready to transform to female phase.

Male phase. Soon after hatching from the egg, and in one species even before hatching (Moritz, 1939), a slender copulatory organ, the phallus, grows out from the body behind the right tentacle (Figs. 1, 2, 5). During copulation the phallus transfers the sperm from the seminal vesicles of the male to the female's vagina, whence they reach the uterus and the seminal receptacles (Figs. 4, 6).

Transition phase. After the completion of the

rudiment which may completely disappear in the female phase (Figs. 1, 2, 4, 5, 6).

During the period of sexual transformation, the ovogonia and young ovocytes, which have remained on the wall of the gonad while spermatogenesis was in progress, resume activity. In species in which there is a sharp demarcation between male and female phases, as shown in Fig. 7, the discharge of the sperm and the subsequent cytoly-

of the residual cells leave only a few ovogonia d scattered ovocytes along the walls of the nadal follicles (Fig. 7). The individual has thus urned to a state of sexual immaturity.

Female phase. At the beginning of the female ase the ovogonia multiply rapidly, and the ovotes increase in size to such an extent that the sulting ovary is many times larger than the when young, now becomes strictly sedentary; she receives her mate, lays her eggs in capsules beneath her foot, and protects her young until they are prepared for their own independence.

Although all normal individuals pass through this sequence of sexual phases, the extent of each phase may be profoundly modified by environmental conditions such as unfavorable temperaturel

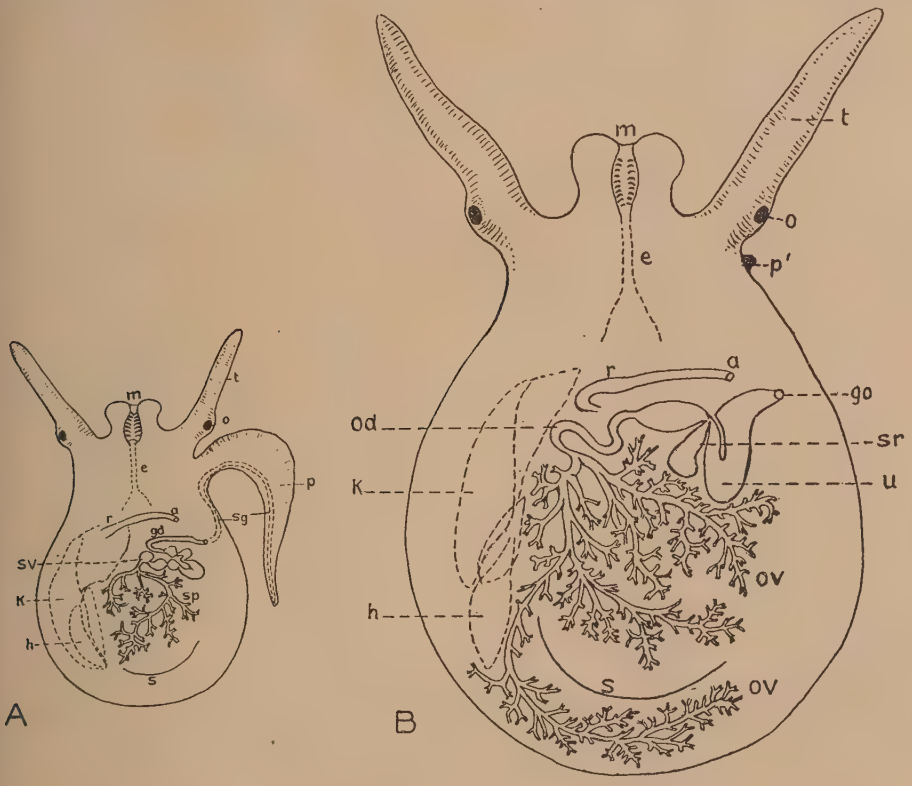

FIG. 4. CONSECUTIVE SEXUALITY IN CRUCIBULUM SPINOSUM

Diagrams of reproductive organs in male and female phases, drawn at the same scale of magnification. A, male ıase, showing branching spermatic follicles (*sp*), seminal vesicles (*sv*), seminal groove (*sg*) and phallus (*p*); other ıtters indicate: *a*, anus; *e*, esophagus; *h*, heart; *k*, kidney; *m*, mouth; *o*, ocellus; *s*, internal shell; *t*, tentacle. , female phase, with ovary (*ov*), oviduct (*od*), uterus (*u*), genital opening (*go*), seminal receptacle (*sr*) and rudient of former phallus (*p'*); other letters as in A.

ɔermary which it has supplanted (Fig. 7, C'). 'he genital duct is then reconstructed into oviduct, terus with fertilization chamber and seminal reeptacles, and vagina (Figs. 4–6). In the meanme the individual may have grown to several ımes the size it had attained while the male phase ras in progress.

Associated with the change in sexuality is a ıarked alteration in behavior, whereby the indiidual, which was so characteristically masculine lack of suitable food, parasitism, accumulation of wastes or other deleterious substances in the water, and especially by association with other individuals of the same species.

This influence of association has been observed in several different species. Gould (1917a, 1917b, 1919) came to the conclusion that the intimate association of the young individual with another of larger size, but of either sexual phase, was necessary for the realization of the functional male phase

in *Crepidula plana*. Young individuals, if isolated, were thought to remain in a neuter condition until their later transformation to females. The male-differentiating stimulus was supposed to be transferred through the water from an older (particularly female-phase) individual to the sexually undifferentiated young. Ishiki (1936, 1939) came to the same conclusion in regard to two other species of the genus.

It has been shown by Coe (1935, 1936a, 1936b, 1938a, 1938b), however, that neither in *Crepidula plana* nor in any of the five other species of that

There seems to be no evidence that the accentuation and prolongation of the male phase by association with the female is due to any masculinizing chemical secretion or hormone which might pass through the water from the body of the female to that of the male, as was formerly assumed. It seems more probable that the stimuli received by the male through his sense organs, during his association with the female, are of such a nature as to influence his masculine characteristics, presumably by the mediation of hormones secreted within his own body (Coe, 1938b).

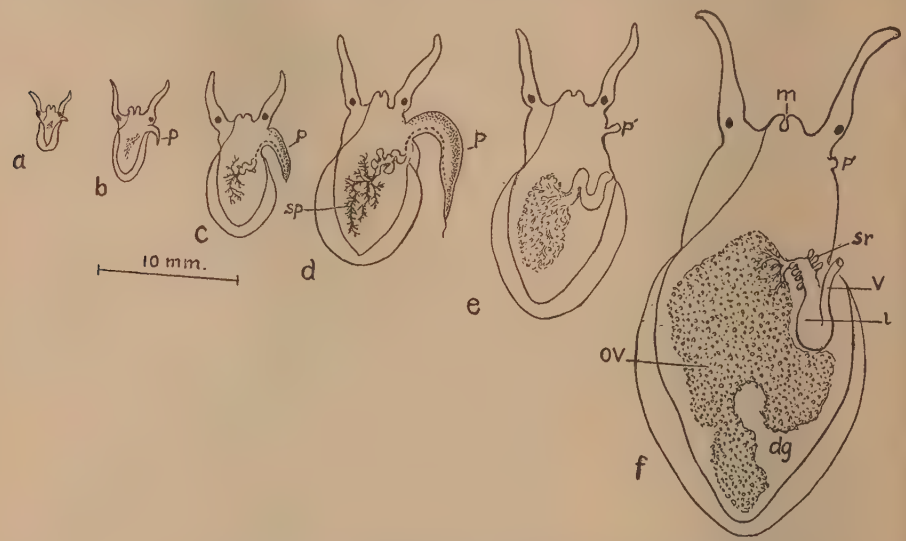

FIG. 5. CONSECUTIVE SEXUALITY IN CREPIDULA ONYX

a, b, immature male-phase individuals with developing phallus (*p*); c, d, functional males with fully developed phallus (*p*) and spermary (*sp*); e, transition stage, with spermary transforming to ovary and phallus (*p'*) nearly absorbed; f, mature individual in functional female phase, with ovary (*ov*), uterus (*u*), vagina (*v*), seminal receptacles (*sr*) and a small rudiment of the former phallus (*p'*). All figures drawn at same scale.

genus studied is association with other individuals a prerequisite for the development of the functional male phase. Such association, although usual in nature, is not an essential stimulus for maleness. This is true also for *Crucibulum spinosum* (Coe, 1938c).

Nevertheless, the association of the male-phase individual with one in the female phase, with the accompanying opportunity for copulation, not only prolongs the period which the individual will spend in the male phase but also increases the amount of sperm produced. Isolation of male-phase individuals during the mating period usually leads to the termination of that phase and the inauguration of the female phase.

The sequence of sexual phases in these mollusks is comparable with other types of metamorphosis in animals and is likewise subject to environmental influences. Either the male or the female phase can be accelerated, postponed, or inhibited experimentally, but the sequence cannot be reversed (Coe, 1938a).

It should be mentioned however, that not all the young male-phase individuals react in the same way to the mating stimulus. Some appear to be less masculine than others in their behavior. They leave their mating positions after a few weeks and move to other situations on the female's shell or to neighboring objects. They then begin transformation to the female phase while much younger

l smaller than other individuals which, in some cies, may retain the mating position for several nths or even for more than two years (Coe, 8b).

t has often been observed that the male phase omes functional at a smaller size in mated than olitary individuals. Although it has sometimes is usually followed by rapid growth, the substance of the cytolyzed male gonad, gametes, and phallus being utilized for the nourishment of the somatic tissues of the following female phase. Some of the young, either by chance or by choice, remain solitary throughout the entire male phase. Minor genetic differences may be responsible for varia-

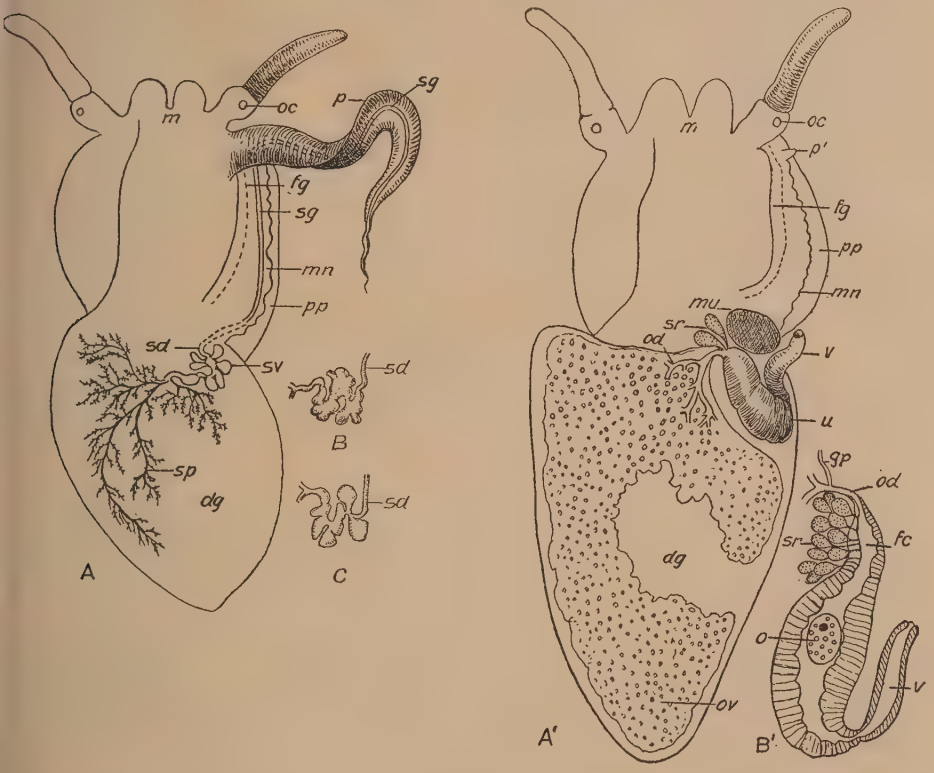

FIG. 6. CONSECUTIVE SEXUALITY IN CREPIDULA ONYX

A, male-phase individual with fully developed phallus (*p*), spermary (*sp*), seminal vesicles (*sv*) and sperm groove), the latter leading to end of phallus. B, C, variations in conformation of seminal vesicles and sperm duct (*sd*). individual in female phase, after transformation of the gonad into ovary (*ov*) and the genital duct into oviduct '), uterus (*u*), vagina (*v*) and seminal receptacles (*sr*). B', optical section of uterus with ovum (*o*), fertilization amber (*fc*), seminal receptacles (*sr*) and vagina. Other letters indicate: *dg*, digestive gland; *fg*, food groove; gonopericardial duct; *m*, mouth; *mn*, mantle; *oc*, ocellus at base of tentacle; *p'*, rudiment of former phallus; propodium.

en assumed that this is the direct result of ating, it seems more plausible to suppose that ose young, which reach the male phase earliest, e the ones that are most likely to be mated rliest, by virtue of the early development of their asculine instincts which lead them to seek the nale. Furthermore, the rapid development of e reproductive system tends to retard the growth the body temporarily. Isolation of mated males tions in the activities of the gonads and, thereby, for differences in behavior under apparently uniform conditions.

In *Crepidula fornicata* and *C. onyx*, those individuals which live on muddy bottoms, where there are no solid objects to which they can attach themselves, frequently pile up in groups of 6 to 12 or more. These groups continue from year to year, newly arrived young attaching themselves

to the top of the group as the old, female-phase individuals die at the bottom (Fig. 8).

When the individuals composing these groups are separated, the oldest females will be observed to have lost the ability to attach themselves to any

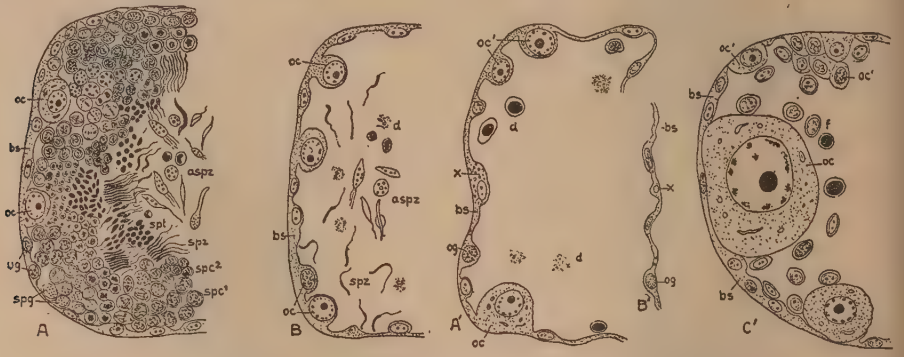

FIG. 7. CONSECUTIVE SEXUALITY IN CREPIDULA FORNICATA; TRANSFORMATION OF THE GONAD FROM MALE TO FEMALE PHASE

A, section of gonad in functional male phase, showing maturation of spermatozoa (*spz*) and atypical spermat somes (*aspz*), with two ovocytes (*oc*) on periphery. B, early phase in sexual transformation; most of the spe matogenic cells have been discharged. A′, transition to female phase; multiplication of ovogonia (*og*) and grow of ovocytes (*oc*). B′, wall of completely emptied follicle. C′, early female phase with proliferating ovogenic ce (*oc′*), follicle cells (*f*) and one nearly mature ovum (*oc*).

object and soon perish, because of their inability to feed or as the result of attacks by enemies. The younger individuals tend to collect in new groups, again with the females at the bottom and the young males at the top (Coe, 1938a).

Immature young of both *C. fornicata* and *C. onyx* often attach themselves singly to rocks or other solid objects which allow unlimited space for growth. If they chance to be isolated from other individuals, the male phase is relatively brief and growth continues in the transition stage and female phase to a size much greater than is possible when attached to an object so small as to restrict the growth (Fig. 8). The size and shape of the shells often differ so greatly that the two growth forms were formerly thought to constitute separate species (Coe, 1942b). The arrival of a male-phase individual is necessary in order that fertile eggs may be produced, because the sperm, which were formed when the individual was in the male phase, are all cytolyzed before the female phase becomes functional. After mating, an isolated individual may continue to produce fertile eggs for more than a year.

The ratio of male-phase to female-phase individuals is much larger when the animals are associated in colonies than it is when individuals are

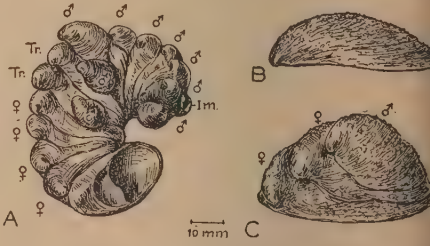

FIG. 8. CONSECUTIVE SEXUALITY IN CREPIDULA ONYX

A, group of 16 individuals living on muddy botto at the base are 4 female-phase individuals to which tw transition-stage individuals are attached; superimpos on these are nine male-phase individuals and one whi is sexually immature. The basal female is attached a dead shell of the same species. B, isolated individu which had attached to rock; such individuals experien only a brief male phase and reach a much larger si than when they are attached to small objects. C, grou of two female-phase individuals and one superimpos male; the basal female had been attached to rock. drawings at same scale.

isolated, for the reason that the length of tim which is spent in the male phase is greater in th former case than in the latter.

A few representatives of other families have similar sequence of male and female phases,

Ankel (1930) has demonstrated in *Janthina* an *Scala*.

Consecutive sexuality is also found in sever genera of the suborder Pteropoda, including *Aplysia, Clio, Clione, Cymbelia,* and *Eolis*. The pr tandric nature of the reproductive system is ve

finite, the gonads of nearly all young individuals ing distinctly of the male type, while the adults nction as hermaphroditic females. This type of xuality has been fully described by Hsaio (1939a, 39b) for *Limacina retroversa*.

In some species of slugs (family Limacidae) the nctionally hermaphroditic condition is likewise eceded by a brief male phase. The sexuality of e gonad appears to be influenced by the nutrition the animal, for when nourishment is abundant ere is a much larger proportion of ovocytes as mpared with the spermatogenic cells than under ss favorable conditions. Starvation checks the oduction of ovocytes, and the gonad may appear be exclusively male after the individual has en kept for two months without food (Richter, 35).

Rosenwald (1926) also found that *Limax laevis* came protandric or proterogynic according to e environmental conditions, particularly the nount of moisture. The more favorable the nditions were for rapid growth the greater was e proportion of ovocytes in the ambisexual gonad.

3. *Rhythmical consecutive sexuality*

In the discussion of functional hermaphroditism was mentioned that there is often a tendency r the male and female sexual cells to be formed succession and not simultaneously. Not infre-ently the sperm are discharged from the gonad each spawning period before the ova are fully ature.

This type of sexuality is well illustrated by *lvata tricarinata*, as described by Furrow (1935). ere the gonad is divided into medullary and rtical portions, the former producing sperm and e latter ova. The individual is morphologically bisexual, with highly differentiated accessory productive organs of both sexual types. At each productive period the sperm mature first. They e then discharged from the gonad and trans-rred to the seminal receptacle of another indi-dual before ovogenesis is completed. After ovu-ion the gonad returns to its former ambisexual ndition in preparation for another sequence of mporary male and female phases.

4. *Alternative sexuality*

A good example of this type of sexuality is found the limpet *Patella vulgata*. In this species, as orted by Orton (1919, 1928), 70.8 per cent of e smallest, and evidently youngest, sexually mature individuals in the population studied were found to be functionally male and only 4.4 per cent female. With increase in size and age the proportion of males decreases until the largest, and presumably the oldest, individuals showed a ratio of 34.7 in the male phase to 64.2 in the female phase. Direct proof of change of sexual phase was not obtained but the conditions are closely similar to those in the oviparous oysters, where a change of sexual phase in both directions has been definitely established (Coe, 1938d, 1943). Orton suggested, as has since been proved for the oviparous oysters, that the young population contains two kinds of male-phase individuals, true males and temporary males (protandric females).

Most of the other species of true limpets (group Docoglossa), however, appear to be of separate sexes, although *Acmaea rubella* is ambisexual (Thorson, 1935), and a few other examples of ambisexuality have been reported.

B. UNISEXUALITY; DIOECISM; GONOCHORISM

Except for the numerous cases noted in the preceding sections, all the other species of the order Prosobranchiata have been thought to be of separate sexes. It is not improbable, however, that further study of the life histories of the 12,000 or more species included in the order will yield many additional examples of ambisexuality. In some of the species the males can be distinguished externally not only by the phallus but also by their more slender shells. When fully adult they are commonly smaller than the females of the same age and may have different habits during the reproductive season. In some species they also have shorter lives. For these reasons great inequalities in the sex ratios of small as compared with large individuals in any collection should not be considered as satisfactory evidence of a change in the sexuality of any individual (Van Cleave and Chambers, 1935; Pelseneer, 1935; Gallien and de Larambergue, 1938; Cole, 1942).

In several families of prosobranchs there are neither morphological provisions nor instincts for mating; the phallus is absent and the reproductive cells of both sexes are discharged into the water. The continuation of the species depends upon the maturity of eggs and sperm at the same season of the year and also upon the stimulus which the presence of gametes of one sex provides for the activation of the spawning reaction in individuals of the opposite sex in the vicinity Many of the

species belonging to the suborder Scutibranchiata have these characteristics.

II. AMPHINEURANS

Most of the representatives of the class Amphineura are of separate sexes but none of them has any provision for copulation. Both sexes, when subjected to appropriate stimuli, discharge their reproductive cells into the water (Grave, 1922). The production of very numerous minute ova and a tendency of the animals to congregate are the only assurances of fertilization. A large chiton (*Ischnochiton*) may deposit nearly 200,000 eggs at a single ovulation. In a few species spermatozoa from neighboring males may enter the branchial furrow or even the oviduct of the female and fertilize the eggs contained therein.

With the exception of *Trachydermon raymondi*, all the species of true chitons (order Polyplacophora) of which the life histories are known are unisexual. This is likewise true of the Chaetodermorpha of the order Aplacophora. All the other wormlike Aplacophora are ambisexual, and in some of them (*Dondersia* and *Neomenia*) the hermaphroditic condition is preceded by a brief male phase (Pelseneer, 1935).

III. SCAPHOPODS

All the representatives of the class Scaphopoda, so far as known, are of separate sexes and are without provision for internal fertilization. The gametes of both sexes are discharged into the water.

IV. CEPHALOPODS

The Cephalopoda are invariably of separate sexes, with highly specialized organs for copulation. The sperm are packed in capsules which the male transfers to the mantle cavity of the female by means of one of his arms. This arm is specially modified for the purpose, and in *Argonauta* and a few other genera, it is detached from the male's body during copulation and left within the female's mantle cavity. The missing arm is later replaced by regeneration.

CONCLUSIONS

Of the five different types of sexuality found in animals and plants, namely, functional ambisexuality, consecutive sexuality, rhythmical consecutive sexuality, alternative sexuality, and unisexuality, all are represented among the mollusks. Both the pelecypods and the gastropods have examples of all of these types, but the majority of species of pelecypods are unisexual while the gastropods are about evenly divided between unisexual and ambisexual species. The amphineurans show all types except alternative sexuality, although most of the species are unisexual. The scaphopods and cephalopods are strictly unisexual.

But little is known regarding the physical hereditary basis of sexuality in the mollusks, for only a few doubtful examples of heterochromosomes have been reported (Perrot, 1930). Several cases of environmental influence on the expression of sexuality in ambisexual species and on the sex ratios in unisexual species have been mentioned in a preceding section but no experimental proof of phenotypic sex determination has been obtained.

Certain species both in the pelecypods and in the gastropods are characterized by local races some of which are unisexual and others ambisexual and various species of gastropods may be either oviparous or ovoviviparous, according to the locality. Further experimental evidence is needed to determine the extent to which these differences can be influenced by environmental conditions.

In those cases of consecutive sexuality and rhythmical consecutive sexuality that have been mentioned in this and the preceding review (Coe 1943), the primary sexual phase may be either male or female but it is usually male. Histological studies show that the developing gonad is more or less distinctly ambisexual, and that one of the two alternative sexual types of primary gonia continues to multiply, while the other type is usually inhibited. In a small proportion of individuals both types remain active and the young animal is functionally hermaphroditic. In the succeeding sexual phase either one type or the other may become dominant. In consequence there may be several simultaneous variants of the usual male female-male sequence in a single population, as is well illustrated in the pelecypods by *Teredo navalis* and the larviparous oysters (Coe, 1943). Some individuals may be unisexual and others ambisexual.

In some of these cases it is histologically evident that the primary phase is female only when the usual male phase is aborted. Such an abortion can be produced in the gastropod *Crepidula* by subjecting the young individual to unfavorable conditions for a time and later returning it to more suitable environment. The hereditary male tendency of the young individual appears to be

pited until the time when the physiological litions for femaleness have been reached. The arous oysters sometimes respond in a similar ner.

all cases of consecutive sexuality, rhythmical ecutive sexuality, and alternative sexuality, individual must be determined genetically for the alternative phases of sexuality, with asso-ed factors that determine which of the two es shall be activated first. If the primary e is to be male, as it usually is, the activity of spermatic tissues is associated with the devel-ent of such specialized efferent ducts, seminal cles, and copulatory organs as may be charac-tic of the species. Associated also are instinc-patterns of behavior peculiar to the male.

ne inference may follow, although without ite proof, that the primary gonad releases stimuli as are necessary to activate first the ordia of the accessory sex organs and later nervous mechanism associated with behavior. is, in general, in harmony with the observed logical differentiation of the organs.

was mentioned that, in species of *Crepidula Crucibulum*, the length of time that the indi-al remains in the male phase is influenced by conmental conditions, such as available nutri-and particularly by opportunity for copula-The male phase can be terminated experi-tally either by an unfavorable environment or solation. But in all normal individuals the phase will come to an end under even the best itions and this will generally, but with some otions, occur at approximately the same age the mated members of the population. The or size, at which the sexual transformation will ually begin varies with the species and is pre-bly conditioned upon a particular state of iological maturity. There is doubtless an litary basis. There are also individual differ-in the behavior of various young members of population, for some of them remain solitary, r by chance or by choice, and experience only f male phase. Perhaps these differences may ise be referred to various hereditary modify-actors.

e male phase, in species with consecutive lity, is unstable and indicative of immaturity t may be long continued in an appropriate onment, such as is furnished by a suitable which provides satisfactory stimuli. When stimuli are removed or become ineffectual, the le sexual phase ensues.

As stated in a previous review (Coe, 1943), all these manifestations of sexuality are consistent with the view that the prospective sexuality of the individual is dependent upon the quantitative balance of the male, as opposed to the female, factors in the hereditary mechanism. They may all be referred to the homozygosity or heterozygosity of the primary sex factors, with their associated modifying factors for the activation or suppression of either the male- or female-determining components at certain stages of development or in harmony with different degrees of physiological maturity. They are also responsive to environmental conditions.

Finally, the influence of internal secretions on sexuality may be considered. The presumably universal occurrence in vertebrates of hormonal substances necessary for the development and functional activity of the sex organs would seem to imply that secretions of a similar nature may be present in invertebrates. But as yet conclusive evidence to that effect has been found only in crustaceans and insects. There seems to be no satisfactory evidence that any substance of such a nature passes from an older to a younger individual, either in *Crepidula* or any other gastropod, as was formerly supposed to be the case and is so stated in many textbooks. There is no reason to believe that association is a prerequisite for either of the two sexual phases in any mollusk with consecutive sexuality.

In most invertebrates there is only a single set of primordia for the accessory sex organs, and these are generally homologous in the two sexes. But the organs which they form may differ greatly, presumably because the activating agency in the male, or in the male phase, is of a different nature from that in the female and activates different parts of the primordia. Each part appears to be attuned to a specific stimulus. In some of the mollusks, however, the copulatory organs of both sexes, but particularly of the male, have quite diverse origins.

In individuals with consecutive sexuality the parts peculiar to the primary sexual phase may be reduced to a simplified or neutral condition at the time of sexual transformation. The system is later redifferentiated by the agencies of the succeeding sexual phase in harmony with the functional requirements of that phase. In hermaphrodites certain parts of the primordia become differentiated into organs of the male type at the same time that other parts are forming the female organs.

Functional activity, however, is dependent upon agencies additional to those which govern the differentiation of the organs; there must be mutual interaction of the organs themselves with other secreting and reacting systems, including the nervous system. Only then is physiological sexuality realized, and only then do the sexual instincts become operative.

Functional sexuality, therefore, requires the participation of a long series of hereditary reaction systems which are activated one after the other in orderly sequence under the influence of suital external and internal conditions. The enviro mental requirements may change during successi stages of development. Each developmental eve as it occurs is a response to a preceding action a in turn initiates the one that is to follow. If a one of these reactions fails to occur, either becau of hereditary or environmental deficiency, fu tional sexuality is not realized, and the individ is incapable of sexual reproduction.

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Contribution No. 229

UTILIZATION OF SEAWEEDS

By C. K. TSENG

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UTILIZATION OF SEAWEEDS*

By C. K. TSENG

QUISITIVE children often ask their elders t the heaps of rotten plants washed re on the beach. A typical answer is, , they are seaweeds.'' To most persons enjoy swimming or strolling along the hes seaweeds are very unwelcome. Prob- when the early Latin scholars, such as il and Horace, spoke of these plants as *lior alga,*'' they had the same feeling too, ould there be anything more worthless noxious than the seaweeds which spoiled otherwise clean and lovely beaches? In when a Roman had said that something more worthless than an ''*alga,*'' he had ably gone as far as the Latin language d carry him!

he thrifty Orientals, however, have quite fferent attitude toward marine growths. ough they too regard most of the marine ts as weeds, they call certain kinds ''sea tables.'' The latter are so highly es- ed as food that natural production is sufficient to satisfy the demand for them consequently they are cultivated like n crops. Moreover, some marine algae long served as medicine and others have employed in sizing silk and for various r purposes. To those who know the e of marine crops, it seems inconceivable the useful forms should have been called ''weeds.''

the last two centuries utilization of sea- ds has been gaining favor in the Western d. While the food-conscious Orientals been using luscious marine plants fly for subsistence, the industrially- ded Occidentals have developed other . Previously they were burned to form ash from which soda, potash, and iodine extracted. Now they are processed to d algin, agar, carrageenin, and other use- products. Few realize, however, that the ted States is annually deriving millions ollars worth of valuable products from e humble plants.

ome eighty years ago, E. C. C. Stanford, the discoverer of algin and one of the pioneers in the promotion of seaweed industries, addressed the Royal Society of Arts in London on the economic applications of algae. The following statement made by the chairman after the lecture could represent the opinion of those who have come to realize the value of marine plants:

> It had been remarked by those who noticed the progress of invention that language generally had to undergo changes corresponding with the changes of the times. Now he, for one, wished that the word ''weed'' should cease to be applied in the indiscriminate manner it was to marine plants. Hitherto ''weed'' meant a useless or noxious plant. Mr. Stanford had shown that a vast proportion of marine plants, so far from being ''weeds'' in the ordinary conception of the word, were extremely useful ''vegetables''; and he hoped they would come before long to limit the word ''weed'' to those marine plants which were worthless, or which were not capable of being turned to accounts.

Unfortunately, there is as yet no simple word to take the place of ''seaweed'' in its general sense so that we might differentiate between the useful and the useless plants of the sea. Illogically we still have to use the term ''seaweed'' in the same indiscriminate way that was criticized almost a century ago, unless we want to use the more technical combination, ''marine algae.''

To people who have never enjoyed the taste of some delicious sea vegetables it may seem inconceivable that seaweeds or their products could be eaten. Nevertheless, they are unconsciously consuming certain seaweed products, which have, in recent years, entered into our daily diet. To mention some examples: many types of confectionaries, canned goods, the so-called health foods, and practically all ice cream and chocolate milk contain seaweed extracts.

Uses of Seaweeds

The chief uses of seaweeds may be classified under the following five categories: foods, drugs, gums, chemicals, and stockfeed and fertilizer.

Foods. More than one hundred kinds of

Contributions from the Scripps Institution of ography, New Series, No. 229.

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FIG. 1. *LAMINARIA JAPONICA**

YOUNG JAPANESE KELP PLANT FROM CHEFOO, CHINA. A COMMON FOOD SEAWEED ON THE ORIENTAL MARKETS.

marine plants are utilized as food by the Chinese, Japanese, and Hawaiians. Of these kelp and purple laver are consumed in larger quantities than all the others combined.

The edible kelps in the Orient are species of *Laminaria, Undaria,* and *Ecklonia.* The most important is *Laminaria japonica* (Fig. 1), which is known in China as *haitai* and in Japan as *kombu.* The Japanese probably eat their kelp as regularly as the Americans eat their lettuce and tomatoes. The Chinese do not consume as much kelp as the Japanese, because China produces only small quantities of it. Annually, she has imported about 55 million pounds of *haitai* from Japan, and in spite of its foreign origin, it has been sold in the inland provinces thousands of miles from the ports of entry.

The purple lavers are members of the genus *Porphyra,* called by the Chinese *tsuts'ai* and the Japanese *amanori.* The demand for this group of sea vegetables, espe-

* Figures 1, 2, 3, 6, 7, and 8 are reproduced through the courtesy of Professor Wm. Randolph Taylor, University of Michigan.

cially *Porphyra tenera* (Fig. 2), is so gre that natural production both in Japan a in China is not enough to meet the deman Consequently, it is extensively cultivated Japan, where a total area of over twel thousand acres is devoted to *amanori* far ing. Annually over 65 million pounds this seaweed, valued at about 9 million y (about $3,000,000), were produced, some which was exported to China.

Scientists do not entirely agree on the fo value of seaweeds. Experiments have sho that some seaweed carbohydrates are n easily digested by humans because of t absence of suitable enzymes in the alime tary tract. Research on others indicates rather high degree of digestibility. We st do not know enough about the actual nut tional value of seaweeds. Possibly, like t land plants, some have a high nutritive val whereas others have very little or even non

Seaweeds are comparable to lettuce a celery. Whether they have high nutriti value in the sense of supplying energy a building tissue is of secondary importan Because they are stimulating or appetizin

About one-fourth actual s

FIG. 2. *PORPHYRA TENERA*

THE PURPLE LAVER OF THE ORIENT, ANOTHER COMM EDIBLE SEAWEED ON THE MARKETS OF THE FAR EAS

use they supply necessary salts in read-
available organic form, and because they
ish vitamins, seaweeds should have a
e among common foods.
is possible that a seaweed diet may be
eventive for hay fever. Although plants
ng more virulent pollens than those from
American ragweeds are found to occur
apan and coastal regions of China, yet
e areas have few hay fever cases. But
ntals are not immune to such allergy;
act, many of them become hay fever vic-
after having stayed in the United States
some time. It is generally accepted that
ssium chloride may help to alleviate suf-
ng from hay fever. Since the Japanese
the Chinese eat many kinds of seaweeds
contain large amounts of potassium
ride, it is probable that the custom of
ng seaweeds may be responsible for the
pression of hay fever in the Orient.

rugs. The Chinese *Materia Medica* has
mmended, from early days, the use of
veeds, especially the kelps and the sar-
sums, in the treatment of goiter and other
ndular troubles. These plants have also
n used in the treatment of dropsy. The
ient peoples, of course, did not know why
n seaweeds were so effective in curing
se maladies. They found out the special
ne of the humble marine plants merely

About one-half actual size

FIG. 3. *DIGENEA SIMPLEX*

M CHINA. AN IMPORTANT ORIENTAL DRUG PLANT
M WHICH MACNIN AND HELMINOL ARE EXTRACTED.

About one-half actual size

FIG. 4. *GELIDIUM AMANSII*

A YOUNG CYSTOCARPIC SPECIMEN OF THE PRINCIPAL AGARPHYTE IN THE ORIENT. FROM TSINGTAO, CHINA.

by trial and error. Now, the curative principle is known to be iodine.

A well-known drug on the Oriental market is the tropical seaweed, *Digenea simplex* (Fig. 3), known in China as *Tserkoo-ts'ai* or *Haijen-ts'ao.* In recent years the Japanese have prepared a drug from it called *macnin.* A pharmaceutical under the name of *helminol* also comes from the same source. It is said to be as effective as the standard vermifuge, santonin, and, because it has no secondary bad effect, especially on children, it is rated as better.

Gums. Seaweed gums are hydrophilic colloids which form mucilaginous sols or firm gels in water at low concentration. Agar, algin, carrageenin, and funorin are the four kinds of gums made from seaweed sources. Because of their peculiar properties, they have been used extensively as stabilizers, deflocculants, textile sizers, and semielastic media. Their commercial importance has increased rapidly in recent years.

Agar is extracted principally from species of *Gelidium* (Fig. 4). Although agar in the

About one-fifth actual size

FIG. 5. *MACROCYSTIS PYRIFERA*

YOUNG GIANT KELP PLANT, FROM WHICH ALGIN IS MADE IN CALIFORNIA. SPECIMEN FROM LA JOLLA.

form of a jelly has been eaten by the Orientals for ages, it was not until the middle part of the seventeenth century that the process of preparing its dried form by a simple freezing and thawing method was accidentally discovered in Japan. As a stabilizer and a gel-forming substance agar has numerous applications. In some of its special uses there is as yet no successful substitute. Since the outbreak of the war it has attracted considerable attention in this country. More than 90 per cent of the agar consumed in the United States formerly came from Japan. Since the war the California agar industry has greatly increased its production and is now making enough agar for essential uses in this country.

The most important service that agar renders to mankind, in war or in peace, is as a bacteriological culture medium. In dentistry it serves as an impression mold. Its extraordinary water-holding ability makes it valuable in wound dressing and as a laxative. In the photographic industry it replaces gelatine for certain types of films. In the hot drawing of tungsten wire agar is used in making a special lubricant to facilitate the operation and to reduce the wear on the die. It is also extensively used in bakery products, in clarifying liquids, in making health foods, in confections, and in ice cream. Recently it has been used in the manufacture of marine storage batteries.

Algin is derived from the giant kelp, *Macrocystis pyrifera* (Fig. 5), on the West Coast, and from various species of *Laminaria* (Fig. 6) on the East Coast as well as in England, France, and Japan. The term generally refers to the water-soluble salts of alginic acid, especially sodium alginate. It was discovered in 1883 by the English chemist, E. C. C. Stanford.

The important use of algin is as a stabilizer in the manufacture of ice cream. It is also a valuable agent for thickening, emulsifying, suspending, and coating various sub-

About one-fifth actual size

FIG. 6. *LAMINARIA SACCHARINA*

FROM WEST BARNSTABLE DUNES, MASS. A VALUABLE EAST COAST KELP FOR THE EXTRACTION OF ALGIN.

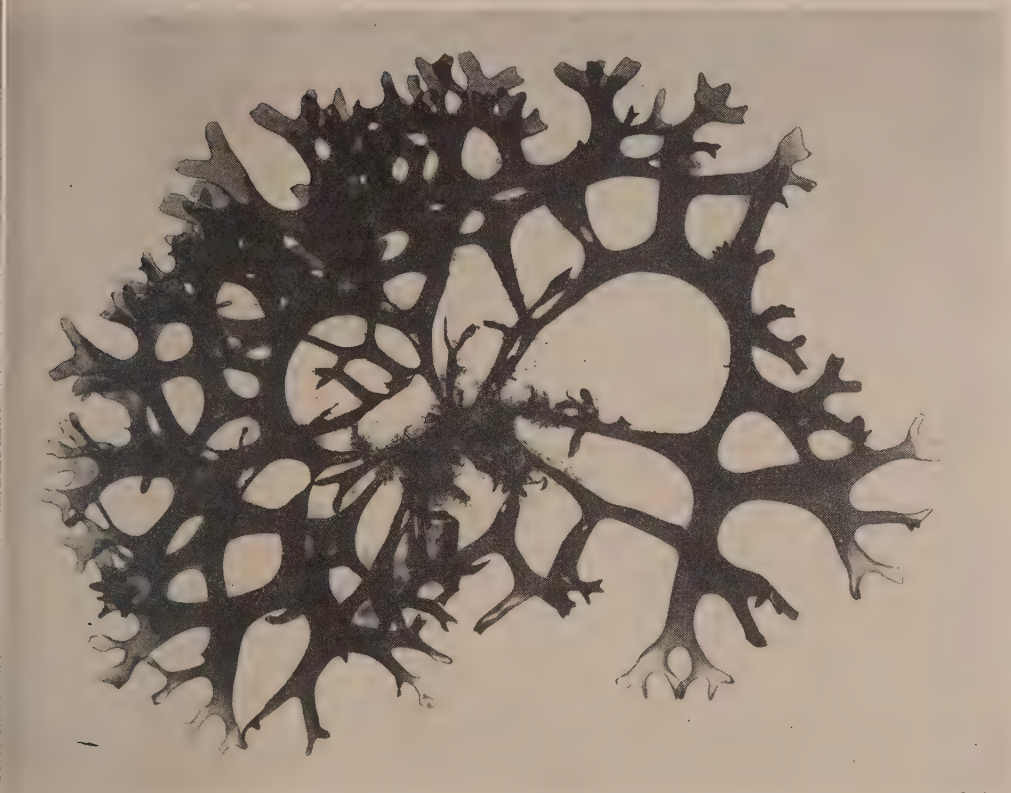

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FIG. 7. *CHONDRUS CRISPUS*

THE WELL-KNOWN IRISH MOSS OF THE ATLANTIC COASTS, A SOURCE OF THE GUMLIKE SUBSTANCE, CARRAGEENIN.

tances. It is used in camouflage paints, re-proofing compositions, latex coating for ignal wires, and water-treating compounds. t is also utilized in making pharmaceuticals. Recently discovered is its use as a coating or oilproof paper containers.

Carrageenin is the cold-water-soluble extract from Irish moss, botanically *Chondrus crispus* (Fig. 7). The seaweed itself was used extensively and exclusively, in earlier times, to prepare the well-known dessert, blancmange. About a hundred years ago the United States used to import large quantities of this seaweed from Europe at a cost of as much as two dollars a pound. It was later discovered that the same plant grew in great abundance in the cooler waters of the Atlantic Coast. For the past century, Scituate, Massachusetts, has been the center of the American Irish moss industry. Lately, large quantities have also been harvested in Maine. A recent estimate placed the production of *Chondrus* on the Atlantic Coast at about five million pounds per annum. It is generally agreed that Irish moss extract is one of the best agents for stabilizing the chocolate fibers in making chocolate milk.

Funorin is the gluey substance in the seaweed *Gloiopeltis* (Fig. 8), known as *funori* in Japanese and *hailo* in Chinese. Commercially, the entire, dried, frequently bleached seaweed is used without extracting this gluey principle from the plant. It is readily soluble in lukewarm water and may be identical with, or at least closely related to, the Irish moss extract. It is mainly used in sizing textiles, especially silk, and in this respect is said to be better than carrageenin. Japan produces about four and a half million pounds annually. China, although the second largest producer, also imports some from Japan to supplement her insufficient supply.

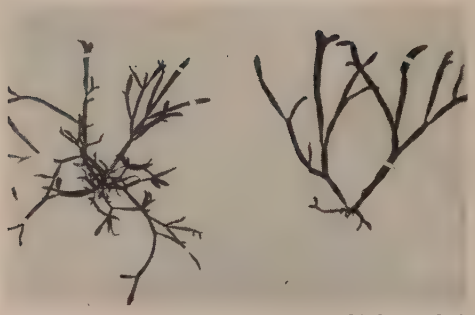

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FIG. 8. *GLOIOPELTIS FURCATA*
FROM CHEKIANG, CHINA. IN THE ORIENT A GLUE OBTAINED FROM IT IS USED FOR SIZING TEXTILES.

Chemicals. During the eighteenth century one of the chief sources of potash and soda in Europe was seaweed ash. At the beginning of the nineteenth century, Scotland alone produced annually about twenty thousand tons of kelp ash, worth about forty thousand pounds sterling. When the manufacture of soda from common salt commenced early in the nineteenth century, the value of seaweed ash dropped rapidly. With the discovery of iodine in 1812, the value of kelp gradually increased until 1873 when iodine began to be recovered from the mother liquors obtained in the manufacture of sodium nitrate from *caliche*. The kelp industry then declined for the second time. The first World War stimulated the revival of the kelp industry in the United States and in other countries as a source of badly needed potash, which used to be a German monopoly, as well as iodine, acetone, and calcium acetate. Soon after the cessation of hostilities, the kelp factories folded up, and the industry declined for the third time. At present potash, iodine, and other chemicals are still being produced from kelps in some countries, but in the United States it is no longer profitable to do so.

The seaweeds utilized in the manufacture of chemicals are the kelps, the fuci, and their related forms. In Europe and Japan they are chiefly species of *Laminaria, Fucus,* and *Sargassum,* and on the Pacific Coast of the United States, the giant kelp, *Macrocystis pyrifera,* is the most important one.

Fertilizer and stockfeeds. Whole or chopped seaweeds are widely utilized as fertilizers in countries with extensive coast lines, especially China, Japan, Great Britain, and France. These plants contain not only large quantities of soluble potassium compounds and some nitrogenous compounds, but also bulky organic substances which slowly decay in the soil and form humus. The seaweeds used for manures are generally the large ones, especially members of the genera *Laminaria, Fucus,* and *Sargassum.* In Ireland the demand for seaweed manure is so great that one species, *Fucus vesiculosus,* is specially cultivated to meet the demand. In most cases the seaweeds are collected by farmers in the vicinity of the seacoast and applied by them directly to their own fields. Because of its high potash content, seaweed fertilizer is especially beneficial to root crops such as the potato, sweet potato, and beet. As a fertilizer it is fully as valuable as barnyard manure and has the added advantage of being free from weed seeds and fungus spores.

In certain European countries herds pasturing along the seashore graze on living fuci and other algae exposed at low tide. Seaweeds are gathered, boiled, and mixed with meal, and the resulting mixture fed to horses, pigs, and cattle. In California a kelp company prepared a kelp meal from *Macrocystis,* which was found to be a good food supplement for chickens, foxes, and cattle.

The Seaweed Industry in California

California has one of the richest coasts in the world—not only in fish resources but also in marine vegetation. The value of the relatively young seaweed industry in this State is already worth at least two million dollars annually, with only a rather small portion of the potential natural production being utilized.

Agar. This extract is the only one from seaweed sources that has received the attention of the War Production Board. Two months after the attack on Pearl Harbor, this commodity was frozen and its uses restricted to the preparation of bacteriological culture media.

In California agar is extracted from the local seaweed, *Gelidium cartilagineum,* which occurs in commercially harvestable quanti-

es from Point Conception southward. It a reddish-purple, fern-like alga which rows on rocks from the low tide mark to a epth of thirty or more feet. It grows best n turbulent waters and in regions where here is a continuous swell. Although it may ttain a height of four or more feet, it is arvestable when about one and one-half feet ll. Practically all the commercial weeds re harvested with a diving rig, only a small uantity being gathered with a long-handled ake. The diver wears a complete diving utfit and, crawling over the rocks, pulls the eed off by hand. In a good working day f about six diving hours, an experienced iver can get as much as a ton of the wet eed. Generally, however, the amount harested per day is between 1,000 and 1,500 ounds. There are not many working days n a year, since during the winter months he ocean is usually too rough for the diver work. Even in the summer, there may e only two days out of three when the water calm enough for this rather strenuous and recarious work. The weed is dried in the n and baled, but it is not bleached, as is he practice in Japan.

The California method of processing agar much more modern than the traditional apanese procedure, but the basic principle the same in both: agar is soluble in boiling ater but not in cold water. Thus, when rude agar gel is frozen and later thawed, he cold water draining away will carry with the soluble salts, pigments, and various impurities. Agar may also be precipitated by lcohol and other chemicals, although this ethod has not yet been applied commerally. The freezing-thawing method seems ill to be the cheapest one, although technologists are working on improved methods. The first agar factory in the Western Iemisphere appeared in Glendale (formerly ropico), California, about 1920. Strange say, the person who founded an industry hich eventually freed the United States rom dependence upon Japanese agar, was Japanese, Chokichi Matsuoka. He proc-ssed the weeds by the traditional Japanese rocedure, with some minor modifications. he company apparently was not financially uccessful and was later sold to a group of ative Americans. It remained for a Californian, the late John Becker, to modernize the agar manufacturing method. Now there are agar factories at San Diego, South Pasadena, Los Angeles, Orange, and Whittier, all in southern California. In the largest of these factories, producing about one-third of the quantity of agar previously imported by the United States from Japan, the process is as follows: The agarphyte is soaked in cleaning vats and then the agar is dissolved from the plants by hot water in pressure cookers (Fig. 9). The hot solution passes through filter presses to tubs where it gels upon cooling. The resulting gel is crushed and is poured down into cans in the freezing room (Fig. 10). The frozen gel is thawed and the cold water, in which impurities are dissolved, is separated from the agar particles in rotary vacuum filters. As the resulting agar flakes still carry about 90 per cent water, they are delivered to stack driers (cylinders three stories high) in which they are dried by an ascending stream of hot air.

Algin. The giant kelp, *Macrocystis pyrifera,* provided the United States with potash during the last war. It is now producing another important article, algin, which is likewise helping the war effort. While algin is also being produced on the East Coast, from *Laminaria,* more than 70 per cent of the total U. S. production comes from southern California.

Macrocystis is truly a giant kelp, growing to a length of over one hundred feet. It attaches to rocks at a depth of ten to seventy-five feet in regions where there is a continuous swell. Thanks to the private and government investigations conducted during and shortly before the first World War, we have a better knowledge of this seaweed than of any other useful form. At present the harvesting of kelp is highly mechanized and is effected by a mowing machine, cutting three to four feet below the surface, and carrying as much as three hundred tons in a single load. The operation is carried out in calm weather throughout the year.

The method of extracting algin is based on the fact that alginic acid itself is insoluble whereas its sodium salt is readily soluble in water. The fresh kelp is therefore digested with soda, which reacts with the acid to form

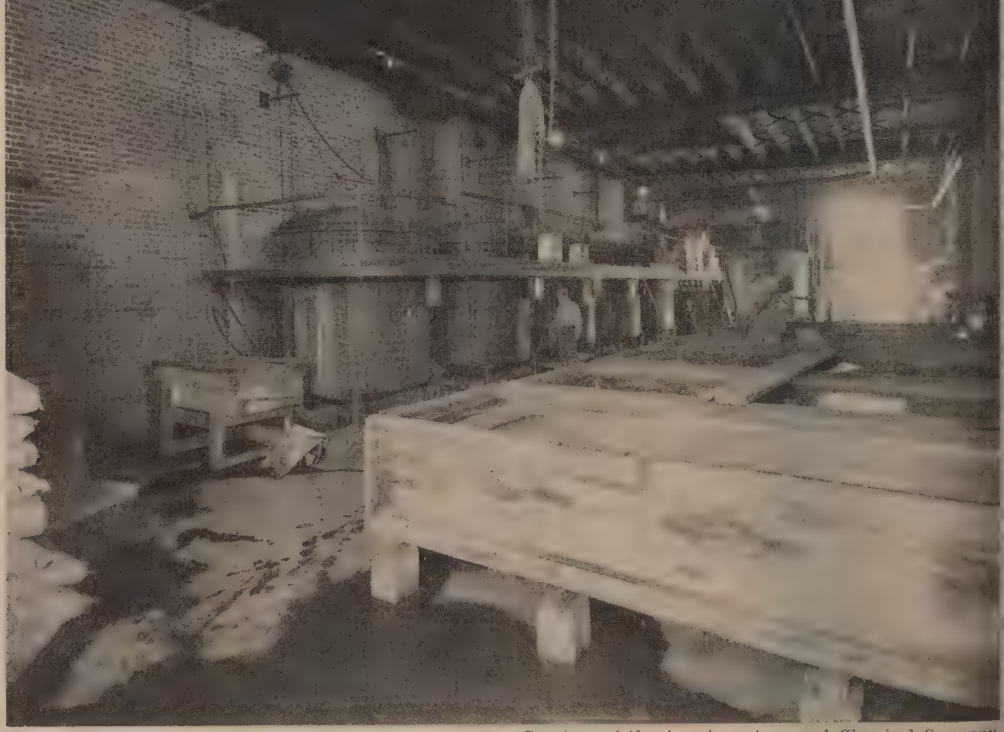
the soluble sodium alginate. This is filtered from the pulpy residue, and the subsequent addition of an acid precipitates the insoluble alginic acid. A wide variety of metallic and organic salts of alginic acid are marketed.

Porphyra. The only seaweed which has been utilized directly for food in any quantity in California is the perforated purple laver, botanically *Porphyra perforata.* This is a very common seaweed along the entire California coast. Being an annual, it generally appears in winter and spring and continues to grow until early summer. It grows on rocks in rather sheltered places to the limit of high water.

It was reported that 300,000 pounds of dried *Porphyra* were harvested in 1929 by white men and Indians in northern California and by Chinese in central and southern California. Chinese buyers distributed the products to Chinese stores or exported them to China, especially to Hong Kong and Canton. The Chinese harvesters usually came to the *Porphyra* grounds in the fall and burned, with driftwood, the rocks on which they expected their seaweed crop to grow. By early spring these rocks were densely covered with the laver. In harvesting, the seaweed is merely pulled from the rock by hand and is dried in the sun in the form of rectangular sheets.

The Future of the California Seaweed Industry

It has previously been remarked that the amount of useful seaweeds now being harvested in California is only a small fraction of the total natural production. The greatest obstacle to the development of the local



Courtesy of the American Agar and Chemical Company

FIG. 9. INITIAL TREATMENT OF AGARPHYTES FOR PRODUCTION OF AGAR

SOAKING VATS ARE ON THE RIGHT, PRESSURE COOKERS ARE ON THE LEFT, AND FILTER PRESS IS IN THE REAR

Courtesy of the American Agar and Chemical Company

FIG. 10. ROOM IN WHICH PARTIALLY PURIFIED AGAR-GEL IS FROZEN
HE CANS FILLED WITH CRUSHED AGAR-GEL ARE LOWERED INTO THE COLD BRINE BENEATH THE SLATTED FLOOR.

eaweed industry lies in the high cost of the aw material, because of the necessity of sing human labor in the harvesting. 'hanks to the efforts of scientists, especially he late Professors W. A. Setchell and N. L. ardner of the University of California, we ave a good knowledge of the kinds of sea- eeds that grow on the Pacific coast, but we ave neglected other phases of research on eaweeds, especially the relationship between he seaweeds and their chemical and physical nvironments, the life history of the red sea- eeds, the development and growth of the seful species, and the technology of seaweed roducts. Consequently, we are still unpre- ared to build a great seaweed industry in 'alifornia.

For several reasons the processing of kelp ndoubtedly has a more promising future ıan that of other seaweeds: First, we have better knowledge of the essential biology of *Macrocystis* than of any other useful seaweed. Second, scientists have estimated that we could harvest annually more than 30 million tons of fresh kelp south of Point Conception. As the kelp companies in southern California are probably harvesting only about fifty thousand tons annually, the supply of the raw material for the future expansion of the industry is practically unlimited. Third, the demand for algin as a stabilizer is continuously increasing, and new uses for it are also being found. Finally, the harvesting of kelp has been highly mechanized, thus reducing greatly the cost of the raw material.

The agar industry faces a much more difficult future: First, we still know very little about the growth, reproduction, physiology, and life history of the agarphyte, *Gelidium cartilagineum*. Second, the harvesting of this seaweed must be effected entirely by manual labor and below the surface of the

sea. Furthermore, it is not easy to collect because it generally grows in small patches and usually clings to the ragged edges of rocks. A practical mechanical device to harvest it has not yet been invented, although thousands of dollars have been spent in the attempt. As a result, the cost of fresh *Gelidium* is almost fifty times that of fresh kelp. Third, the threat of Japanese competition after the war makes American interests reluctant to invest money in an industry which may collapse in the face of cheap foreign imports. In some past years it has been reported that the agarphyte harvested in California cost almost as much as the finished agar imported from Japan. Fourth, although California agar is much more carefully and scientifically manufactured than the Japanese product, our present manufacturing method still needs numerous improvements. For instance, a significant portion of the agar now remains in the discarded pulp after cooking—a portion which should be recovered.

During the present war emergency there is no Japanese competition, and the government is buying agar at three dollars a pound. After the war, however, conditions may be very different. If the California industry cannot produce a cheaper agar, it will be doomed to failure. In fact, in its twenty-three years of existence the American agar industry has persisted only through periodic injections of new capital and has several times been at a standstill. Fortunately, through the persistent effort of a few persons like the late John Becker, and also because of the high quality of the local product, California had, at the outbreak of the war, a skeleton agar industry. In the interest of future national defense, the agar industry should be encouraged to attain a permanent status. This can be achieved in part by research on the biology of the principal agarphyte, *Gelidium cartilagineum,* and also by research on the technology of manufacture. Another possible solution of the problem is to find other agar-bearing seaweeds which can be gathered at a lower cost than *Gelidium.* Recently it was found that *Gracilaria confervoides,* one of the seaweeds used in agar manufacture in other countries, occurs in large quantity in bays in southern California. From this plant, a much cheaper agar can be produced, because of the relatively low cost of harvesting it. The Gracilaria-agar seems to be poorer in quality than the Gelidium-agar; however, it should be useful as a substitute for the latter in certain fields. South Africa and Australia are already producing agar-gel from *Gracilaria.* England is also reported to be trying to make her own agar from a local seaweed, probably the same plant. On the East Coast, some Gracilaria-agar has already been produced and offered for sale. Utilization of the West Coast *Gracilaria* will greatly increase the local production of agar and may also help to reduce its cost.

When Americans have come to realize that some seaweeds, *Porphyra* for instance, are palatable and just as delicious as some of the vegetables, the seaweed food industry will certainly have a promising future. At present the consumption of *Porphyra* as food in this country is limited to Orientals, especially the Chinese. "Wild" purple laver is abundant on the coast of California. Moreover, this plant can always be cultivated without much difficulty and on a large scale if the natural supply is not sufficient to satisfy the demand.

Besides *Macrocystis, Gelidium, Gracilaria* and *Porphyra,* California has many other kinds of potentially useful seaweeds. For instance, there are several species of *Gigartina* and *Iridophycus* which contain a seaweed gum very similar to, and probably identical with, carrageenin extracted from Irish moss. Large quantities of these medium-sized seaweeds are found in various places on the Pacific Coast, especially in the latitude of Monterey. I once gathered from the rocks about eighty pounds of *Gigartina corymbifera* in only fifteen minutes at low tide.

In order to achieve a successful, permanent seaweed industry in California it is highly desirable to carry on further research on the biology and life history of the useful species, on the ways and means of utilizing them, on the harvesting methods, and on the technology of extracting the unique substances present in these marine crops.

Contribution No. 230

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NOTES ON THE ALGAL GENUS TAENIOMA[1]

C. K. TSENG

The algal genus *Taenioma* (Delesseriaceae, Rhodophyceae) was founded by J. G. Agardh (2, p. 1256) on *Polysiphonia perpusilla* which he (1, p. 16) himself previously had described from material collected by Liebmann at St. Augustin on the Pacific coast of Mexico. The then monotypic genus was characterized by (1) a filiform, dichotomously branched frond with polysiphonous, articulate, noncorticate filaments; (2) the presence of marginal cells half the length of the segments on the flattened portion of the frond; and (3) dilated, vittiform stichidia bearing tetrasporangia (then known as sphaerospores) in a double series. The genus was placed by its author, together with *Sarcomenia,* in the tribe Sarcomeniae which was then placed in the family Rhodomelaceae but later more properly removed by Schmitz (19) to the Delesseriaceae.

Thuret (7, p. 69, pl. 25) added another species to the genus, namely *Taenioma macrourum,* citing as a synonym *Hutchinsia macrourа* Schousboe in herb. The type material came from Tangier, Morocco, in the Mediterranean. Not having seen J. Agardh's specimen of *T. perpusillum* Thuret, in describing his species, expressed some doubt as to the specific difference between the Mediterranean form and the one from the Pacific. In keeping the two separate, he was probably influenced by the widely separated regions from which the two plants were collected, some apparent differences in size and color, and especially the presence in the Mediterranean plant of two apical hairs which were presumably absent in the alga from the Pacific. It should be noted, however, that J. Agardh, *op. cit.,* did mention the presence of apical hairs in his plant: "Stichidium . . . apice saepe in fila minuta 3 . . . excurrens." Examination of Agardh's type specimen by Howe (*in* Thompson, 21, p. 98) has confirmed this. The writer has also examined a fragment of the original specimen deposited in the herbarium of the New York Botanical Garden and is in perfect agreement with Howe.

Several years after the publication of *Taenioma macrourum* Thuret, Bornet (6, p. 297) examined a specimen of *T. perpusillum* J. Ag. and came to the conclusion that the Pacific and the Mediterranean plants are not separable specifically. Later, Heydrich (12, p. 295) and De-Toni (9, p. 732) adopted Bornet's view. Schmitz and Hauptfleisch (20, p. 415), however, retained both species. In his classical work on the Rhodomelaceae, Falkenberg (10, p. 709, pl. 15, fig. 21–29), devoted some space to the morphology of the Mediterranean plant, which he separated from the Pacific alga chiefly on the basis of its having long monosiphonous

[1] Contribution from the Scripps Institution of Oceanography, New Series, No. 230.

apical filaments on the flattened "leaves," presumably absent in *T. perpusillum* J. Agardh. This, as already pointed out above, is not correct. Falkenberg also mentioned that the two species differed in the nature of the branches. He remarked that in the Mediterranean plant the cylindrical segments of the side branches and also those of the axis between two branches were so short and compact that it was easily distinguishable from the Pacific plant, which was much slenderer and more loosely branched. "While it is true that the Pacific alga has much longer side branches, the shortness and compactness of the segments are similar in both cases. It is no wonder, therefore, that later authors (cf. Collins and Hervey, 8, p. 117; Børgesen, 4, p. 341; and Okamura, 17, p. 26) in trying to differentiate the two species on this basis, could not see the desirability of keeping them apart.

Thompson (21) published a detailed study of the morphology of *Taenioma,* using two different sets of materials collected by Howe at Porto Rico (Puerto Rico) and at West Caicos in the Bahamas. She was probably the first to note some of the important differences between *T. perpusillum* and *T. macrourum* although she discussed both forms under the latter name. Such differences between these two collections as the branching and the number of apical hairs and segments in the flattened shoots have been correctly emphasized. In an editorial note in Thompson's paper, Howe justified that author's identification of the Bahamian plant with the Mediterranean species. He questioned, however, "the identity of this *T. macrourum* with the previously described *Taenioma perpusillum* of J. Agardh . . . ," remarked that his Puerto Rican plant and the type of J. Agardh's species from Mexico are "essentially the same except that the terminal hairs are much longer and more luxuriant in the Porto Rican plant," and concluded that he was "inclined to agree with Bornet . . . with Heydrich . . . and with De-Toni . . . in considering *Taenioma macrourum* (Schousb.) Thur. a synonym of *Taenioma perpusillum* J. Ag."

Howe seemed to have regarded the differences of these two sets of specimens, which he considered to belong to the same species, as a matter of ecological influence. He made the remark that the Bahamian plants "were found growing in an inland pond or lake, having evidently a subterranean communication with the sea—a place where several marine algae of recognizable species were more or less abnormal and peculiar," and the Puerto Rican plants "were growing where they were well exposed to the surge of the open sea." It should be noted, however, that Børgesen (4, p. 341), whose plant was evidently of the same species as Howe's from the Bahamas, found it "in an open place upon reefs of calcareous algae, etc."

To date, phycologists seem to have regarded this problem of the *Taenioma perpusillum-macrourum* complex as satisfactorily

settled, have generally adopted the view of Bornet and his followers, and have conventionally put *T. macrourum* as a synonym of *T. perpusillum* whenever that species is reported.

The third species of *Taenioma* was described by Farlow (11, p. 236) on material from San Diego, California, and was named *Taenioma Clevelandii*. As described, it "has scattered stichidia, is four inches high, and has a striking resemblance to *Griffithsia tenuis* Harv.," and its "stichidial branches terminate in a more or less acute apex instead of two hairs." It was also mentioned that "in the lower part of the frond, the angles between the primary cells are filled with a small but irregular number of secondary cells." All these peculiarities are certainly very different from the characteristics of the *Taenioma perpusillum-macrourum* complex and the writer has long doubted its being a member of this group. He has studied some specimens collected at Moss Beach, Pacific Grove, California (*Hollenberg 3228*) and is fully convinced that it should be separated from *Taenioma*.

Recently Hollenberg (13, p. 534) has pointed out several additional differences between *Taenioma Clevelandii* Farl. and *T. perpusillum* J. Ag., although he still preferred to keep the Californian plant in the same genus. Dr. Papenfuss who has made a critical study of *Taenioma Clevelandii* has agreed with the writer that it does not properly belong to *Taenioma*. He has further decided that it should be transferred to the genus *Platysiphonia* Børg., because (1) its branches are endogenous in origin; (2) it does not form terete branches; (3) it does not have determinate branches ending in hairs; and (4) its tetrasporangial branches have a dorsiventral organization, since the stalk cell of a tetrasporangium forms a large cover cell on one surface and a rudimentary one on the other. These characteristics are so fundamentally different from those of *Taenioma*, that there should no longer be doubt among phycologists that the plant from San Diego should be removed from the genus *Taenioma*.

Some years ago, the writer gathered from Hong Kong a collection of a *Taenioma* rich in tetrasporangia. At first, he was inclined to follow others and consider *T. macrourum* Thur. a synonym of *T. perpusillum* J. Ag. The more the specimens and literature were studied, however, the more hesitant he was to do so. After a thorough study of his collection, which has been preserved in excellent condition for microscopic examination, and the extensive collections of Howe, including some fragments of the type specimen of *T. perpusillum* J. Ag., deposited in the Herbarium of the New York Botanical Garden, the conclusion was finally arrived at, that the differences, especially those reported by Thompson and Howe, do exist, and are very constant. Other differences have also been found. The fact that the same form has been reported to occur in sheltered and exposed places and both forms in more or less similar situations naturally eliminates

the possibility of their being ecological forms, at least, so far as the factor of the relative exposure to surf is concerned. Furthermore, the geographical distribution of *T. perpusillum* and *T. macrourum* gives evidence of their distinctness. When fundamental differences between these two forms are constant and without intermediates, and when these cannot be traced to ecological or geographical influences, then there is no alternative than to accept the two forms as separate, independent species.

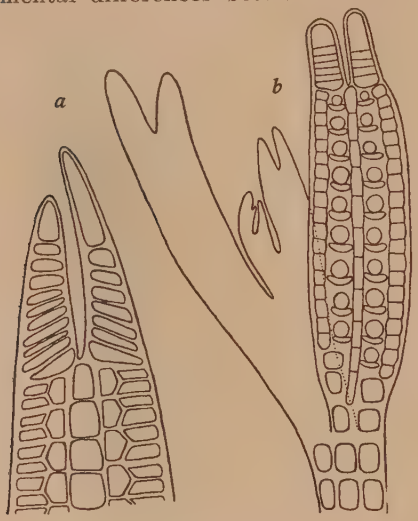

FIG. 1. *Taenioma macrourum* Thuret: *a*, apex of a branchlet showing the two apical hairs, × 560; *b*, upper part of a branch showing a stichidium, × 190.

The most important difference between the two lies in the number of apical hairs. The Puerto Rican and Hong Kong specimens always have three apical hairs on the flattened branchlets and are referable to *T. perpusillum* J. Ag. There are many determinate branchlets which at a glance seem to have only two hairs. A careful study, however, reveals the fact that the oldest hair in the group of three has already dropped off, and the two younger ones are left behind (pl. 25, fig. 5). The Bahamian, Bermudian, and Mediterranean specimens as well as the Japanese plants described and illustrated by Okamura (17, p. 26, pl. 244, fig. 17–19, pl. 245, fig. 5–9, on the contrary, have only two hairs and undoubtedly belong to *T. macrourum* (text fig. 1a). To be sure, Okamura (17, p. 27, pl. 265, fig. 8) mentioned and figured a branchlet with three apical hairs. Whether this was an abnormal form of the normally two-haired plant, or whether Okamura had both species, can be settled only by examination of his specimens which had come from at least two different sources.

The presence of two or of three such apical hairs is neither accidental nor irregular. It is rather a matter of fundamental difference traceable to the behavior of the apical cells of the determinate branchlets. In *T. macrourum,* the apical cell, after having formed the more or less definite number of segments, gives rise by an oblique division to a hair-initiating cell. Later, the apical cell itself assumes the role of hair formation, thus resulting in two hairs (text fig. 1a). In the case of *T. perpusillum,* the apical cell by alternate oblique divisions forms two hair-initials.

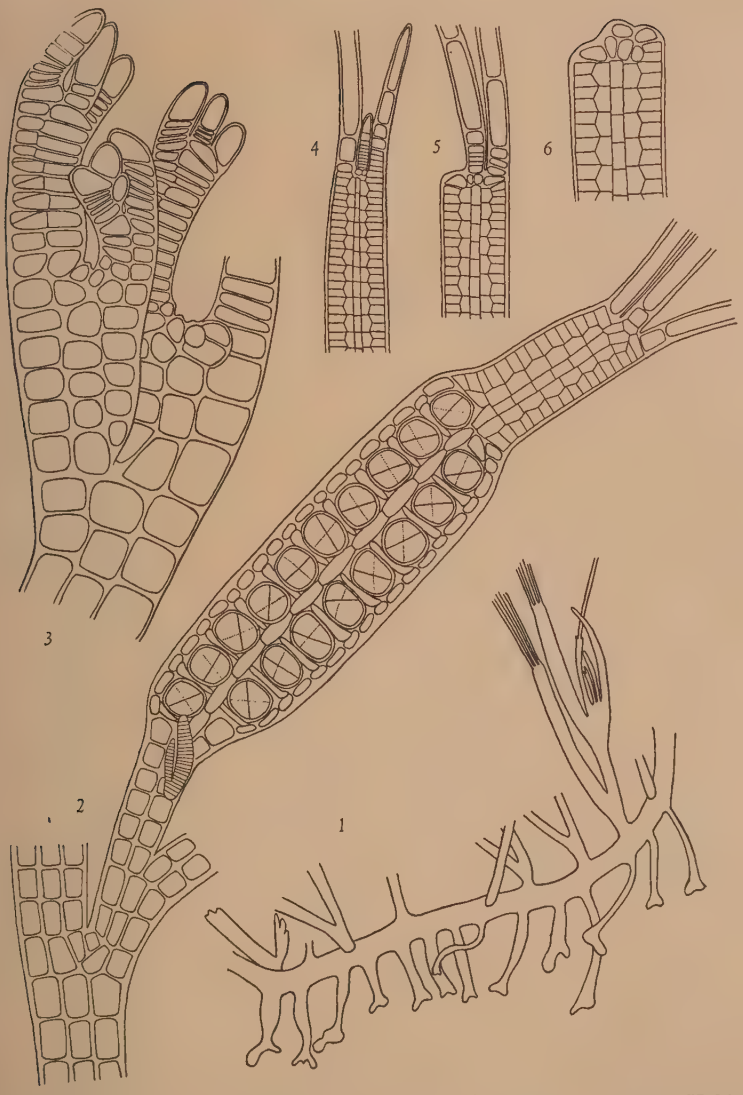

PLATE 25. TAENIOMA PERPUSILLUM (J. Agardh) J. Agardh. FIG. 1. Habit sketch of a portion of a young plant, ×32. FIG. 2. A part of an erect branch, showing a stichidium with tetrasporangia, the differentiation of fertile and sterile portions, and the apical hairs, ×155. FIG. 3. Apical part of a branch showing the apical cells, ×452. FIG. 4. A young branchlet with three hairs in different stages of development, ×155. FIG. 5. An older branchlet with one hair dropped off and two remaining, ×155. FIG. 6. An old branchlet with all three hairs dropped off, leaving three basal cells of the hairs behind, ×155.

One of these develops faster than the other, resulting in two hairs of unequal length. Later the apical cell also gives rise to a hair, resulting in the three terminal hairs characteristic of this species (pl. 25, figs. 2, 3 and 4). In the younger branchlets, while all three hairs are found, the central one generally remains shortest (pl. 25, fig. 4). In older branchlets, the first-formed lateral hair may have dropped off, leaving its basal cell in the original position, while the two which develop later, remain (pl. 25, fig. 5). Then, the other lateral hair and at last the central one drops off, leaving the three basal cells of the original hairs behind. The branchlet at this stage appears stunted, blunt and hairless at the apex (pl. 25, fig. 6). This explains why sometimes there appear to be only one or two hairs, or even hairless forms, instead of the three hairs as described.

Another important difference, already pointed out by Thompson, *op. cit.*, lies in the respective number of segments in the flattened determinate branchlets of these two species. In *T. perpusillum,* there are generally 20 to 35 segments, occasionally as few as 15, and the length of the mature branchlets varies from 0.6 to 1.0 mm. In *T. macrourum,* there are many fewer segments, generally 8 to 20 only, rarely more, and the branchlets are correspondingly shorter, being about 0.25 to 0.40 mm. long when fully mature. In dealing with the length of the branchlets, it is to be noted, the hairs are not included.

There are also observed some differences in the stichidia. In *T. perpusillum,* the stichidium has a cylindrical stalk of 3 to 5 segments followed by the broadened, fertile portion of the "blade" composed of varying numbers of 8 to 14 segments (sometimes as few as 4 and sometimes as many as 17) and then a narrower, sterile portion of 6 to 12 segments upon which are the monosiphonous hairs. Sometimes there are also a few sterile segments between the stalk and the fertile segments (pl. 25, fig. 2). In *T. macrourum,* the cylindrical stalk, with only one or two segments, is followed by the broadened, fertile "blade" and then terminates in the hairs, without an intervening sterile portion as in *T. perpusillum* (see text fig. 1b). This difference between the two species is found in the materials examined. However, in a recent communication Dr. Papenfuss remarks that in his material of *T. perpusillum* the tetrasporangia very frequently extend distally up to the hair-forming segments.

The two species, as they are now separated by the writer, also differ from each other in habit. Generally speaking, *T. perpusillum* is more closely and conspicuously fasciculate, the branching often fastigiate and the branchlets usually alternately disposed, whereas in *T. macrourum,* the branchlets are more loosely disposed and usually secund. In the former species, the erect branches are much taller, about 2 to 3 or more millimeters high, and have as many as twelve determinate branchlets. The latter species has

the erect branches about 1 mm. or less high, with generally 4 to 5 determinate branchlets each.

The original description of the genus by Agardh and the subsequent descriptions of others such as Schmitz and Hauptfleisch are far from complete. Many of the important facts about this group of plants, especially with regard to the sexual organs, were discovered much later. It is Thompson (21) to whom we must turn for our present knowledge of the development of the male organs and the general characteristics of the cystocarps; unfortunately there is no detailed account of the developmental phases of these structures. Collins and Hervey (8, p. 117) mentioned that they found a cystocarp which was, however, lost before a drawing and a description could be made.

By bringing together the collections studied by previous authors and studying these in connection with our own, the following revised description of the genus is made possible.

Taenioma J. Agardh (1863). Plants dorsi-ventral, with horizontally creeping, cylindrical, segmented, polysiphonous, ecorticate, main filaments rhizomatous, giving rise to stout, unicellular rhizoids below and erect branches above. Erect branches arising at rather regular intervals, secundly or alternately, pseudodichotomously branched. Determinate branchlets with short, cylindrical, polysiphonous segmented stalks and flattened, segmented, distal portions. The flattened portion with a distinct midrib of a central siphon surrounded by four pericentral siphons. The dorsal and ventral pericentral cells smaller and the lateral ones larger, each giving rise to two marginal cells, which are very regularly, transversely arranged at the periphery. Apical parts of the determinate branchlets provided with colorless, monosiphonous filaments (hairs). Apical cells large, dome-shaped, dividing by transverse walls. Growth monopodial. Branching exogenous. Tetrasporangia originating from lateral pericentral cells, with two rudimentary cover cells, tetrahedrally divided, in two rows, one on each side of the midrib of the fertile branchlets. Spermatangial branchlets flattened, similar to the tetrasporic ones, fertile portions broadened, spermatangia developed upon cells between axial and marginal cells. Cystocarps urceolate with a terminal ostiole, developed from modified branches.

The general habit of members of this genus is like that of *Herposiphonia* of the Rhodomelaceae, with a horizontally creeping filament giving rise to erect branches at rather regular intervals. The division of the apical cells and the mode of growth are similar to the genus *Caloglossa*. The apical cell repeatedly cuts off disc-like cells below until a more or less definite number is reached and then divides by an oblique wall into two unequal parts (pl. 25, fig. 3). The upper part continues as the apical cell of the filament while the lower, dividing by an oblique transverse wall perpendicular to the first, gives rise to another dome-shaped

cell which becomes the apical cell of the new lateral branch. It follows that the branching is strictly lateral, rather than dichotomous as claimed by J. Agardh, although it may appear subdichotomous. Each segment consists at first of a single disc-like cell, which soon gives rise to two cells on the right and left by two longitudinal divisions, thus forming three cells of similar shape and size in the same plane. Then, two other longitudinal divisions occur, parallel to each other, but perpendicular to the first divisions, thus giving rise to two more cells, one dorsally and one ventrally. The segment thus formed is similar to a typical *Polysiphonia* in having a central cell surrounded by four pericentral cells. The segments of the main filaments, the axis of the branches and the stalks of the determinate branchlets always remain this way. In the distal part of the determinate branchlet, however, the two lateral cells each again undergoes two divisions: first an oblique wall cutting off a triangular cell in the upper part and then a second division cuts off a similar cell in the lower part, thus forming two marginal cells of exactly half the height of the pericentral cells.

In the formation of the tetraspores, each of the lateral pericentral cells undergoes a transverse division, the upper cell becoming the sporangium and the lower one the stalk cell. The sporangium enlarges greatly, still always retaining protoplasmic connection with the stalk cell, and, dividing thrice: a longitudinal division parallel to the flat surface, a transverse one and a third perpendicular to both divisions, give rise to a tetrahedrally divided sporangium (pl. 25, fig. 2). The stalk cells eventually form two cover cells on both surfaces.

Falkenberg (10) mentioned and figured what he suspected to be "antheridia." These are now known to be malformations. Schmitz and Hauptfleisch (20) were the first to correctly mention the spermatia as occurring between the midrib and the marginal cells. Their development was reported by Thompson (21) and the writer's investigations substantiate her conclusions. Briefly, the marginal cells are the first to divide longitudinally; the outer ones remain as such while the inner become the spermatangial clusters by repeated divisions. Later, the pericentral cells on both sides of the axial cells also become involved. Thus, in the fertile portion, all of the cells between the marginal cells and the axial ones give rise to spermatangia. Either the entire "blade" or a part of it becomes fertile. The stalk and the apical hairs remain sterile.

Cystocarps, so far, have been described only by Thompson, *op. cit.* The writer has found, in the Bermuda material, a single urceolate, ostiolate cystocarp with rather broad base and conspicuous neck. Being among crowded filaments in a permanent mount, it cannot be studied in detail. According to Thompson, the characteristics of the cystocarps are those of the Rhodomela-

ceae into which family she preferred to place the genus. It seems that further investigations with better materials are necessary in order to solve this problem. But since the vegetative characteristics as well as those of the tetrasporangia and spermatangia show much closer relationships with such plants as *Sarcomenia* and *Caloglossa,* the writer is inclined to follow Falkenberg, Schmitz, and others in placing this genus in the family Delesseriaceae. Papenfuss (MSS) has arrived at this same conclusion as to the taxonomic position of the genus.

As mentioned above, the genus with its two species is reported from various tropical and subtropical regions, although such records are rather meagre. It seems likely that its distribution is very extensive in warmer waters. The two species can readily be distinguished from each other by sterile characters alone:

A. Plants to 2–3 mm. high, flattened determinate branchlets with 15 to 30 segments, apical hairs three .... 1. *T. perpusillum*
AA. Plants less than 1 mm. high, flattened determinate branchlets with 8 to 20 segments, apical hairs two ..... 2. *T. macrourum*

1. Taenioma perpusillum (J. Agardh) J. Agardh, Sp. Alg. 2(3): 1257. 1863. *Polysiphonia perpusilla* J. Agardh, Öfvers. Kgl. Svenska Vetensk.-Akad. Förhandl. 4: 16, 1848. *Taenioma macrourum* Thompson *non* Thuret, Bull. Torrey Bot. Club 37: 97, pl. 9, f. 12, pl. 10. 1910. (In part.)

In a note deposited in the herbarium of the New York Botanical Garden, Howe remarked that the type of this species was mostly sterile, differing considerably in habit from his tetrasporic collection (no. *2433*), but a tetrasporic plant in the type collection was similar. The principal material upon which Thompson based her morphological study of the genus (*Howe 2433*) was growing on a stick of wood collected near low-water mark, at Point Borinquen near Aquadilla, Puerto Rico on June 15, 1903. This material is rich in tetrasporic and spermatangial branchlets; occasionally what seem to be young cystocarps may also be observed.

The Hong Kong materials (*Tseng 2857*) were found tufted on an exposed, surf-beaten rock, together with various small algae, such as *Herposiphonia caespitosa* Tseng, *Gelidium pusillum* (Stackh.) Le Jol., in the lower littoral region at Shek-O, Hong Kong I., on July 4, 1940. They are all tetrasporic. The primary filament is about 90 to 110 μ in diameter, with the segments shorter than the breadth, about 60 to 75 μ long. It sends down vigorous, stout unicellular rhizoids, to as much as 75 μ in diameter, separated from the mother pericentral cells by transverse walls, and more or less expanded at the ends (pl. 25, fig. 1). Erect branches arise from the decumbent filament quite regularly at intervals of 5 to 9 segments. Their axes are cylindrical and segmented like the main filament, giving rise to minor branches or branchlets alternately and fastigiately. The determinate branchlets with cylindrical

stalks below and flattened "blades" above, terminate in three long monosiphonous hairs. The stalks are cylindrical and composed of 3 to 4 segments, about 45 μ in diameter. The blades have conspicuous midribs, to about 70 μ broad when sterile and 0.6 to 1.0 mm. long, generally with 20 to 30 segments. The hairs are about 20 μ in diameter below. The stichidia are very much broadened in their fertile portions, to about 110 μ broad, otherwise like the ordinary branchlets. The tetrahedrally divided tetrasporangia are disposed in two rows along the axis, generally 8 to 14, although fewer or more have been observed. They are about 50 μ in diameter when mature (pl. 25, fig. 2).

A specimen collected by Grunow without giving the date or locality, and another from Tongatabu (Friendly Is.) by Dr. Graeffe all belong here. Heydrich (12) reported that his specimen from Batjan I., North Molucca, had two or three hairs on the stichidia. Most probably, he had this species before him. Askenasy (3) records *T. perpusillum* from Western Australia and Reinbold (18) records it from Sumatra, both without description. In view of the wide occurrence of this species in the Pacific, these identifications are probably correct.

This species, as herein limited, is known from the following regions: St. Augustin, Pacific coast of Mexico (type locality); Fiji and Friendly Is.; Western Australia; Molucca Is.; Sumatra; Hong Kong, China; and Puerto Rico, West Indies.

2. Taenioma macrourum Thuret, in Bornet and Thuret, Not. Alg. 1: 69, pl. 25. 1876. (In part.) *Polysiphonia nana* Kützing, Tab. Phyc. 13: 10, pl. 29, fig. e–f. 1863. *Taenioma perpusillum,* as interpreted by Bornet (6), De Toni (9), Collins and Hervey (8), Børgesen (4), Howe (15), Okamura (17), *non* Agardh.

Since Miss Thompson (21) based her conclusions to a considerable extent upon material collected at West Caicos in the Bahamas (*Howe 5708*) it is of interest to consider the character of these specimens in some detail. The material is tetrasporic with the main filaments generally 60 to 75 μ in diameter and with segments as long as broad. The rhizoids are about 30 μ in diameter. Erect branches are short, less than 1 mm. high, arising in intervals of 4 to 6 segments. They are more loosely and secundly branched than those of the above species. The determinate branchlets have short cylindrical stalks of 1 or 2 segments and "blades" about 60 to 75 μ broad and 240 to 400 μ long (excluding hairs), with 8 to 15 segments and terminating in two colorless, monosiphonous hairs about 25 to 30 μ in diameter at the base. The stichidia are quite young, about 80 to 90 μ broad, with short stalks of 1 to 2 segments, and "blades" having all segments fertile and terminating in two hairs like the vegetative ones.

Another specimen (*Hervey 7433*), collected from South Shore, Bermuda, on February 3, 1913, has vegetative characteristics

similar to the above. There is found, among crowded filaments on a semipermanent mount, a cystocarp which is broadly urceolate, about 360 μ broad and 480 μ long, including a neck about 120 μ long. There is another Bermudian specimen (*Phyc. Bor.-Am. no. 1935*), collected by Hervey, "forming a gelatinous mass with other algae, Bigget Island, April 1913." This, according to Howe (14, p. 518) "shows a small Rhodomelaceous plant bearing some slight resemblance to the *Taenioma*." The writer, however, has found a few fragments of the determinate branchlets of *T. macrourum* Thur. sparsely scattered among a large mass of a blue-green alga and the Rhodomelaceous plant Howe mentioned. They are sterile.

Taenioma macrourum has been recorded from the following regions: Tangier, Morocco (type locality) and Naples in the Mediterranean; Canary Is.; Bermuda and West Indies; Japan; ? Cape of Good Hope.

Summary

A general historical, morphological, taxonomic and distributional survey of the delesseriaceous genus *Taenioma* J. Agardh is given. *T. perpusillum* (J. Agardh) J. Agardh, described from the Pacific coast of Mexico, and *T. macrourum* Thuret, from the Mediterranean, have been regarded, in the past, as belonging to the same species. The conclusion is reached that they represent two distinct species. Important differences are found in the number of apical colorless monosiphonous hairs, the number of segments in the determinate leaf-like branchlets, the stichidia, and the habit and branching of the erect branches. Geographically, both species seem to be widely distributed and are represented, at least in one instance, in the same region (West Indies). A new locality for *T. perpusillum*, namely, Hong Kong on the China coast, is reported.

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Scripps Institution of Oceanography,
University of California, La Jolla,
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Contribution No. 231

STUDIES ON THE CHEMICAL PRESERVATION OF WATER SAMPLES*

By

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During the storage of water samples collected from the sea, rivers or lakes, increased bacterial activity causes changes in the organic, oxygen, carbon dioxide, ammonia, nitrate or phosphate content or the H-ion concentration of the water (Waksman and Carey, 1935; ZoBell and Anderson, 1936). These changes occur even near 0° C, although refrigeration tends to retard bacterial activity. Consequently, chemical preservatives are frequently added to water samples which are to be analyzed for any of the aforementioned constituents or properties unless the water is to be analyzed soon after collection. Likewise plankton samples are often preserved for later observation.

While the choice of a preservative depends upon the constituent to be determined, in general it should be colorless, miscible with water, chemically inert, preferably volatile or readily neutralized, and of such a nature that it will not interfere with the analytical procedures to be used for the examination of the water. This report is concerned with a study of the relative efficiency of certain preservatives which are widely used or recommended for preventing bacterial activity in samples of sea or fresh water.

EXPERIMENTAL

In the first series of experiments oxygen consumption was used as a criterion of bacterial activity in samples of sea water treated with different preservatives. The work of Bronfenbrenner, Hershey and Doubly (1939) has established that oxygen consumption is a satisfactory method of evaluating the toxic or preservative action of disinfectants. The amount of oxygen consumed is more or less directly proportional to the number of bacteria present and their rate of

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respiration under given conditions (Johnson, 1936). The respiration of bacteria and allied micro-organisms is not influenced by the oxygen tension of the water until it is reduced to less than 0.3 mgm of oxygen per liter (ZoBell, 1940). If the bacteria are not respiring, it is doubtful if they are activating any other chemical changes.

The water samples were collected in 5-gallon carboys, thoroughly shaken to insure uniformity in composition and siphoned into 145 ml glass-stoppered bottles. Preservatives were added to some of the bottles and some were left untreated as controls. Sterile controls were prepared either by heating the water to 124° C for 15 minutes, or by passing it through a fine Mandler filter, after which it was reoxygenated prior to siphoning into glass-stoppered bottles. The dissolved oxygen content of the water was determined by the Winkler method at the beginning of the experiment, and other bottles were analyzed for oxygen after different periods of incubation in the water bath at 22° C. Representative results obtained with some reagents commonly used as preservatives are summarized in Table I.

TABLE I—Oxygen Consumed by Bacteria in 20 Days at 22° C in Sea Water Treated with Different Preservatives

Treatment	*Initial oxygen content*	*Oxygen content after 20 days*	*Oxygen consumed in 20 days*
	mgm/l.	*mgm/l.*	*mgm/l.*
None (control)	7.56	4.41	3.15
0.1% Toluol	7.62	5.16	2.46
0.1% Phenol	7.04	6.30	0.74
0.1% Tricresol	7.59	6.92	0.67
0.1% Formaldehyde	6.69	6.28	0.41
0.5% Chloroform	7.50	7.37	0.13
0.2% Carbon disulfide	7.01	4.34	2.67
1.0% Ether	6.76	3.92	2.83
Heat sterilized	7.36	7.36	0.00
Mandler filtered	7.48	7.45	0.03

The data indicate that none of the reagents in the concentrations used, with the possible exception of chloroform, could be relied upon to preserve samples of sea water for 20 days. However, further experiments have demonstrated that some of the reagents are effective in higher concentrations or for shorter periods of time. This is shown by the data in Table II. Similar results have been obtained with various samples of sea water, as well as with samples of fresh water. The results have been substantiated by experiments in which

bacterial multiplication, nitrate reduction and ammonia production have been employed as criteria of bacterial activity.

DISCUSSION OF RESULTS

Although toluol is commonly used as a preservative for biological materials (Tauber, 1937), it is not satisfactory for the preservation of water samples. Toluol is only slightly soluble in water, but even when its solubility and dispersion have been increased with alcohol or ether it does not prevent the growth of all water bacteria. In fact, certain micro-organisms oxidize toluol. Störmer (1908), Gray and Thornton (1928) and Tausson (1929) have isolated organisms from soil which utilize toluol, and Grant and ZoBell (1942) have reported the occurrence of bacteria in the sea which obtain their carbon and energy requirements from toluol. There was no bacterial activity in water saturated with a mixture of toluol dissolved in chloroform, but the mixture was little more efficacious as a preservative than chloroform alone.

TABLE II—Amount of Oxygen Consumed by Bacteria in Sea Water Enriched with 10 p.p.m. of Peptone after Different Periods of Incubation at 22° C

Treatment	*Oxygen consumed after:*			
	1 day	*2 days*	*5 days*	*10 days*
	mgm/l.	*mgm/l.*	*mgm/l.*	*mgm/l.*
None (control)	2.13	5.70	8.04*	—
0.10% Phenol	0.03	0.38	1.26	5.41
0.25% Phenol	0.00	0.08	0.94	1.60
0.50% Phenol	0.00	0.00	0.00	0.00
0.10% Formaldehyde	0.04	0.36	1.89	4.67
0.25% Formaldehyde	0.00	0.00	0.00	0.00
1.00% Ether	1.72	4.91	7.82*	—
5.00% Ether	0.26	1.07	2.21	4.39
0.50% Chloroform	0.00	0.00	0.07	0.08
0.10% Toluol	0.89	5.27	7.84*	—
0.20% Carbon disulfide	2.29	3.92	5.40	7.86*
0.10% Thymol	1.41	4.38	7.97*	—
Heat sterilized	0.00	0.00	0.00	0.00

* All of the dissolved oxygen was consumed.

One per cent phenol kills most bacteria in a few minutes at room temperature, and 0.1 per cent phenol usually inhibits growth. However, some soil and water bacteria are very resistant to phenol. According to ZoBell, Grant and Haas (1943), a few marine bacteria

have been observed which multiply in the presence of 1 per cent phenol. Some of them multiply in mineral media containing 0.1 per cent phenol as the only energy source. Phenol-resistant or phenol-oxidizing bacteria have been found in soil by Matthews (1924), Gray and Thornton (1928), Evans and Happold (1939), Vigier (1941) and others. Erikson (1941) studied *Micromonospora* species from lake mud which utilize phenol, cresol, toluol, α-naphthol, naphthalene and paradichlorobenzene. We have also encountered phenol-tolerant micro-organisms in sea water, but in about 95 per cent of the 140 ml samples of raw sea water treated with 0.5 per cent phenol there was no evidence of bacterial activity after 20 days at 22° C. Concentrations of phenol as low as 0.05 per cent are definitely inhibitory but are not dependable preservatives. It is doubtful if there are any micro-organisms which can tolerate 5 per cent phenol, but it is hardly practical to add this much to water samples.

None of the cresols used either alone or in combination have proved to be as good as phenol for the preservation of water samples. Pure cresol dissolves in water only with difficulty, and when emulsified with soaps or alkalis, certain salts are precipitated from sea water, thereby rendering the sample useless for many analyses. Certain micro-organisms tolerate a saturated solution of cresol in water. The oxidation of o-cresol, m-cresol and p-cresol by soil micro-organisms has been reported by Gray and Thornton (1928). Cresol-oxidizing bacteria were found in lakes by Erikson (1941). Repeatedly we have found water samples treated with an excess of tricresol becoming soupy with the profuse growth of micro-organisms, although cresol-tolerant organisms do not occur in all water samples.

Some bacteria multiply and are otherwise physiologically active in water treated with 0.1 per cent formaldehyde (Table II). However, in all of the 34 water samples tried, 0.25 per cent formaldehyde has proved to be a dependable preservative. This concentration reduces the solubility of oxygen in water, and formaldehyde may react with nitrogenous organic matter or interfere with chemical analyses which involve the use of oxidizing agents.

Diethyl ether, chloroform and carbon disulfide usually inhibit bacterial activity in water samples, but there are soil and water bacteria which grow in saturated solutions. Chloroform is the most effective of the three. Various combinations of ether, chloroform and carbon disulfide have proved to be no better than when these reagents have been used alone. However, we have found no bacteria which would grow in a combination of 0.25 per cent phenol and 0.5 per cent chloroform. Apparently the phenol-tolerant organisms which are

encountered occasionally cannot tolerate 0.5 per cent chloroform. As much as 0.8 per cent chloroform can be dissolved in water, but it requires considerable shaking of the sample to get 0.5 per cent chloroform in solution. If not completely dissolved, certain colorimetric indicators are preferentially dissolved in the droplets of chloroform, thereby complicating analytical procedures.

Adding enough hydrochloric or sulfuric acid to water to render it more acid in reaction than pH 1.5 provides for its prolonged preservation, as shown by the data in Table III. This requires approximately 5 ml of concentrated HCl or 2.5 ml of H_2SO_4 per liter of sea water. Considerably less acid is required to preserve fresh water, the amount depending primarily upon the buffer capacity. Acidu-

TABLE III—Oxygen Consumed by Bacteria at 22° C in Sea Water Enriched with 10 p.p.m. of Peptone and Treated with Different Concentrations of Hydrochloric or Sulfuric Acid

Acid added	*Amount added*	*pH of water*	*Oxygen consumed after:*		
			5 days	*10 days*	*20 days*
	ml/l.		*mgm/l.*	*mgm/l.*	*mgm/l.*
None	—	8.23	7.81	—	—
Hydrochloric	2.5	1.73	0.95	2.46	5.16
Hydrochloric	5.0	1.38	0.00	0.00	0.04
Hydrochloric	10.0	1.11	0.00	0.00	0.00
Sulfuric	2.5	1.37	0.00	0.08	0.26
Sulfuric	5.0	1.13	0.00	0.02	0.03
Sulfuric	10.0	0.93	0.00	0.00	0.00

lating the water reduces the solubility of oxygen, but the water samples preserved with acid are suitable for most other analyses. Water samples to be analyzed for oxygen content should be treated with the Winkler reagents shortly after the collection of the water. There are a few micro-organisms occurring in nature, such as *Thiobacillus thiooxidans*, for example, which can grow at pH 1.5, but we have never found any evidence of their activities in the numerous samples of sea water, fresh water and sewage which have been acidulated. Phillips and Hatfield (1941) recommend preserving sewage samples for B. O. D. determinations by lowering the pH and then holding the acidulated samples at a low temperature.

Heavy metals such as mercury, copper and silver salts are generally good disinfectants. While low concentrations usually prevent bacterial activity in water samples, salts of heavy metals render the water unsuitable for most kinds of analyses.

Similarly oxidizing agents such as chlorine or iodine compounds,

permanganate or peroxides materially alter the composition of the water and interfere with many analytical procedures. Substances which are effective only in high concentrations dilute the sample too much or may be too expensive to be practical water preservatives.

The use of detergents including 0.1 per cent Aerosol OT, Dreft, Santomerse or Tergitol 4T failed to enhance the preservative action of any of the reagents listed in Table I sufficiently to recommend the addition of detergents. Some of the detergents produced undesirable precipitates in sea water. Petroff and Schain (1940) and others have shown that the killing power of some antiseptics is enhanced by detergents or by surface tension depressants.

Chloroform, formaldehyde or acid are just about as effective in the presence of organic matter as in its absence, so these reagents can be used to preserve plankton samples. There was no bacterial activity in sea water treated with 5 per cent peptone in the presence of 0.25 per cent formaldehyde, 0.5 per cent chloroform or enough acid to reduce the pH to less than 1.5. The acidulation of water does result in the destruction of certain calcareous structures of plankton organisms.

CONCLUSIONS

Toluol, tricresol, thymol, carbon disulfide and diethyl ether are not dependable preservatives for water samples to be examined for constituents or properties which may be altered by bacterial activity.

If they are not objectionable for other reasons, either 0.5 per cent chloroform or 0.25 per cent formaldehyde can be used to preserve water samples.

Nearly all water samples can be preserved indefinitely by the addition of 0.5 per cent phenol, but occasionally micro-organisms are encountered which live in the presence of 1 per cent phenol. When supplemented by 0.5 per cent chloroform, 0.5 per cent phenol is a dependable preservative.

Acidulating water to pH 1.5 which requires about 2.5 ml of sulfuric or 5.0 ml of hydrochloric acid per liter of sea water prevents the activity of micro-organisms ordinarily found in water.

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BIOCHEMICAL FOSSILS[1]

By Professor DENIS L. FOX

SCRIPPS INSTITUTION OF OCEANOGRAPHY

RECENT studies in this laboratory, to be reported in some detail elsewhere, have been concerned with preliminary qualitative and quantitative examinations of carotenoid pigments encountered in marine sediments of hundreds or thousands of years' standing. The preservation of a class of ordinarily highly labile compounds over vast ages of time is less surprising when one considers the special conditions which prevail in the buried strata of the ocean floor, *i.e.*, lack of free oxygen, absence of light and perpetual temperatures of nearly 0° C. The added facts that carotenoids as a class are absorbed in the digestive tract of most animals only at low levels of efficiency, and that they are relatively refractory toward non-oxidative biochemical changes would still further favor their gradual accumulation in the ocean floor.

Pigmentary compounds of the plant and animal porphyrin series have been encountered in petroleum, coal and shale oils.[2,3] Similarly, other chlorophyll derivatives, accompanied by carotenoids, and sometimes also in association with fluorescent pigments common to petroleum, occur in long and deeply buried marine sediments.[4,5] These ancient biochromic compounds, together with other oil-soluble substances in whose presence they occur, may be regarded as diagnostic features in the search for biochemical processes in the origins of petroleum and allied natural deposits.

Carotenoids have been reported in moor soils[6] and in muds from lakes,[7,8] marshes[7] and underground caves.[9] Trask[10] has referred to carotenoids in marine

[1] Contributions from the Scripps Institution of Oceanography, New Series No. 232.

[2] R. Lemberg, *Ann. Rev. Biochem.*, 7: 424, 1938.

[3] A. Treibs, *Ann. d. Chem.*, 510: 42, 1934.

[4] D. L. Fox, *Proc. Nat. Acad. Sci.*, 23: 295, 1936.

[5] D. L. Fox and L. J. Anderson, *Proc. Nat. Acad. Sci.*, 27: 333, 1941. (N.B. The published data are in error (p. 335) by a misplaced decimal, thus appearing as 6.0, instead of the correct value of 0.6 mg per cent.)

[6] O. Baudisch and H. v. Euler, *Arkiv. Kemi Mineral Geol.*, 11A, No. 21, 1934.

[7] B. K. Klimov and E. I. Kazakov, *C.R. Acad. Sci. U.R.S.S., N.S*, 16: 321, 1937.

[8] E. Lederer, "Recherches sur les caroténoides des animaux inferieurs et des cryptogames." Lons-le-Saunier, Paris, 1938.

sediments, but little or no emphasis has yet been directed toward the probable ages, history and biochemical significance of these pigmentary compounds in natural deposits.

In the current investigations, standard methods of carotenoid analysis were applied to samples of very fine dark mud collected from various water depths between 100 and 1,100 fathoms, both in the Gulf of California and in the open Pacific Ocean off the coast of Southern California. Oceanic muds, of estimated ages from 15 years up to 2,500 years, yielded golden, pale greenish or dirty yellow extracts, some samples containing as much as 0.20 to 0.30 mg carotenoids per 100 g of original vacuum-dried material. Gulf muds were far darker in color; one core section taken from the 16-foot level beneath the mud surface, beneath 364 fathoms of water (estimated age of the deposit being some 6,000 years) yielded about 0.6 mg of total carotenoids per 100 g of dry mud.[5]

An arresting fact was the consistent finding of a great preponderance of the carotene or hydrocarbon type of polyene over the xanthophyll or polyene alcohol class in mud beneath the first few inches of its surface. The finding of 80 per cent. or more of total sedimentary polyene pigments as carotenes is in striking contrast with the relative proportions of carotenoid types in other marine materials. In finely suspended marine detritus, for example, carotenes may represent from 6 per cent. to 14 per cent. of the total carotenoids.[11, 12, 13] Again, microscopic algae, such as *Prorocentrum micans* and *Nitzschia closterium* yield only about 10 per cent. of their total carotenoids as hydrocarbons.[13, 14] Species of larger marine plants such as *Fucus*, *Dictyota* and *Laminaria* contain respectively only 25.7 per cent., 15.4 per cent. and 4.8 per cent. of their carotenoids as carotenes. This list could be extended. Most marine animals which have been analyzed for carotenoids have been found to yield chiefly xanthophylls. Numerous species of fishes and ophiuroids contain xanthophyll esters as the only carotenoids.[8, 15–19] Species of tunicates, mollusks,

[9] R. A. Beatty, *Jour. Exp. Biol.*, 18: 144, 1941.

[10] P. D. Trask *et al.*, "Origin and Development of Source Sediments of Petroleum," p. 177. Houston, Texas. Gulf Publishing Company. 1932.

[11] D. L. Fox *et al.*, *Bull. Scripps Inst. of Oceanography*, 4: 1, 1936.

[12] Unpublished.

[13] B. T. Scheer, *Jour. Biol. Chem.*, 136: 275, 1940.

[14] N. Pace, *Jour. Biol. Chem.*, 140: 483, 1941.

[15] F. B. Sumner and D. L. Fox, *Jour. Exp. Zool.*, 66: 263, 1933.

crustaceans, echinoderms and coelenterates may be added to the list of marine fauna whose tissues contain carotenoids chiefly, and in numerous instances solely, of the oxygenated type.[8,13,20,21,22,23]

Among the polyene hydrocarbons encountered in marine muds, the preponderant member was β-carotene, with compounds resembling α-carotene in secondary prominence. These were often accompanied by other carotenes with properties closely resembling those of torulene from red yeasts, leprotene from certain acid-fast pathogenic bacteria, or rhodopurpurin and flavorhodin from bacteria of the *Rhodovibrio* group. Small quantities of carotenes with previously undescribed absorption spectra were occasionally found.

Chief among the polyene alcohols were xanthophylls closely resembling zeaxanthin and the spectroscopically similar diatom pigment diatoxanthin described by Strain *et al.*,[23] fucoxanthin and lutein (or perhaps more probably diadinoxanthin from diatoms and dinoflagellates)[23] and small quantities of pigments resembling taraxanthin or dinoxanthin. Prominent in hypophasic fractions also were xanthophylls spectroscopically similar to sulcatoxanthin (peridinin[23]) petaloxanthin and antheraxanthin, while other carotenoids having a single absorption maximum similar to those of glycymerin or astacene were occasionally detected. Xanthophyllic counterparts of the bacterial carotenoids rhodopurpurin and flavorhodin, reported as occurring with the latter in *Rhodovibrio,* were not encountered in marine muds.

Concentrations of total organic matter tend to decrease with age, *i.e.,* depth of burial, of marine sediments.[24] Our preliminary studies indicate that lipids and carotenoids likewise so decrease, the quantities of carotenes undergoing degradation at a rate somewhat parallel to that of total lipids.

Trask[24] estimates that, of the organic matter synthe-

[16] F. B. Sumner and D. L. Fox, *Proc. Nat. Acad. Sci.*, 21: 330, 1935.

[17] F. B. Sumner and D. L. Fox, *Jour. Exp. Zool.*, 71: 101, 1935.

[18] D. L. Fox, *Proc. Nat. Acad. Sci.*, 22: 50, 1936.

[19] R. T. Young and D. L. Fox, *Biol. Bull.*, 71: 217, 1936.

[20] D. L. Fox and B. T. Scheer, *Biol. Bull.*, 80: 441, 1941.

[21] I. M. Heilbron, H. Jackson and R. N. Jones, *Biochem. Jour.*, 29: 1384, 1935.

[22] D. L. Fox and C. F. A. Pantin, *Phil. Trans. Roy. Soc. London, Ser. B.*, 574: 415, 1941.

[23] H. H. Strain, W. M. Manning and G. Hardin, *Biol. Bull.* In press.

[24] P. D. Trask, "Recent Marine Sediments," 440 *et seq.*, Tulsa, Okla. Amer. Assoc. Petrol. Geol., 1939.

sized annually in a column of sea water 2,000 fathoms deep, the proportion deposited in each square meter of the ocean floor amounts to only some 2 per cent. Trask's computed loss of 98 per cent. of total organic matter is estimated by us to be accompanied by a decrease of some 83 per cent. of the original total lipids, and of nearly 87 per cent. of the original carotenoids, *i.e.*, 97 per cent. of the xanthophylls and about 33 per cent. of the carotenes.

Analyses of a core of ocean mud revealed lipids in concentrations of 0.06 per cent., 0.05 per cent. and 0.04 per cent. by dry weight at the 6-inch, 74-inch and 80-inch levels (estimated to be about 600, 7,000 and 8,000 years in respective ages). All three sections yielded chlorophyll degradation products and carotenoids; but an intermediate section taken from the 44-inch level of the same core (4,000 years) yielded similar greenish cleavage products of chlorophyll but no carotenoids. Instead it contained a pigment with blue-green fluorescence, identical in chromatographic behavior and spectral absorption maxima with a pigment recovered from a sample of California crude petroleum,[5] and similar to a porphyrin isolated by Treibs[3] from a California asphalt. This section of the core yielded tenfold the concentration of lipid-soluble substances found at the other levels.

The general preponderance of carotenes over xanthophylls in muds of the ocean floor may arise from several factors: (1) Xanthophylls are more readily degraded than are carotenes by atmospheric or dissolved oxygen;[25] this is especially true of the prominent marine xanthophyll, fucoxanthin and its isomers, which are also especially susceptible to alteration by heat or alkalies.[23] While the foregoing factors would find less application at the bottom of the sea, two other general processes might well contribute to the situation, both there and in overlying waters, namely: (2) The majority of marine animals so far investigated store chiefly xanthophylls rather than carotenes in their tissues; their storing and partial oxidation of polyene alcohols, with fecal rejection of carotenes could bring about a gradual preponderance of the latter class of carotenoid in bottom sediments. Finally (3) there are indications that some microorganisms living in marine muds may contribute polyene hydrocarbons of their own synthesis, and that other such flora may be capable of reducing xanthophylls to compounds of lower oxygen content, or perhaps even to carotenes.

[25] L. Zechmeister, "Carotinoide," J. Springer, Berlin, 1934.

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BIOLOGY OF THE CALIFORNIA SEA-MUSSEL (MYTILUS CALIFORNIANUS). III. ENVIRONMENTAL CONDITIONS AND RATE OF GROWTH [1]

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In the cultivation of oysters, clams and other mollusks, as well as in studies on their rates of growth under natural conditions, it has been frequently observed that certain years are more favorable than others for rapid increment in size. But there has been no satisfactory analysis of the environmental conditions responsible for the observed differences in growth rates. For this reason an experimental study, extending over four years, has been made of the growth of the California sea-mussel at the pier of the Scripps Institution of Oceanography. At this station daily records are made of the temperature of the water and of the numbers of dinoflagellates, diatoms and bacteria present and potentially contributory to the mussels' ultimate food supply. For the temperature records the writers are indebted to Capt. S. W. Chambers; for the data on phytoplankton to Prof. W. E. Allen, and for those on bacteria to Prof. C. E. ZoBell. They also appreciate the technical assistance of Miss Harriet Dunn and Mr. Carl Johnson.

In two previous papers (Coe and Fox, 1942; Fox and Coe, 1943) the writers have presented evidence relative to the normal rates of growth in this species at different seasons and at different ages and sizes, the different rates in the two sexes, the nature of the food materials and the influence of environmental conditions on the rates of growth. It was concluded from these observations that there is a generally positive correlation of the growth rates both with temperature changes and with the abundance of dinoflagellates present in the water. No similar correlation was found relative to the numbers of diatoms or bacteria. It was emphasized however that the correspondence between the size of the dinoflagellate populations and the growth rates of the mussels was not to be interpreted as the direct effect of dinoflagellates as potential food material, since a large proportion of the living dinoflagellates ingested usually pass apparently unchanged through the mussel's digestive tract. Furthermore the total supply of living phytoplankton which the mussel could possibly obtain is estimated to be so small in amount that even if all the constituents could be fully utilized they would furnish less than one-fifth of the mussels' nutritive requirements. The principal portion of the food was shown to consist of finely divided organic detritus, derived from the disintegration of many kinds of marine animals and plants, including both unicellular and multicellular forms (Fox and Coe, 1943).

Continuation of those observations during three additional years has shown that there are wide variations in the mussels' growth rates, not only from month to

[1] Contributions from the Scripps Institution of Oceanography of the University of California, New Series No. 233.

month but also from year to year. It is the object of this paper to record these variations and particularly to present such evidence as has now been obtained as to their causes. For this purpose the changes in the environmental conditions from month to month have been analyzed as fully as possible. Since there are no rivers in the vicinity and the annual rainfall is small, there is but little variation in salinity. The principal effective variables are the temperature and the food supply.

The experimental mussels were kept in wire-screened boxes immersed in the sea below the low-tide level. Since the increment in size was found to vary with the age of the individual under identical environmental conditions (Coe and Fox, 1942), it was necessary to have the same ages represented at all times. This required the addition of young individuals from month to month and the removal of the oldest.

The experiment was continued from January 1940, to January 1944, with the exception of the first five months of 1942. At nearly all times the experimental boxes contained from 100 to 400 or more individuals, each age group being in a separate compartment. The average age remained nearly constant and all were sexually immature. When the individuals of a group were separately numbered, it was found that some grew rapidly for a period and were then overtaken by others; some became leaders for several months, while others remained dwarfs. For statistical purposes it was therefore desirable to follow the growth of 20 or more individuals of each age group. The mean monthly increments in size for all groups are shown in Figures 1 to 4.

In any consideration of the environmental conditions, it must be kept in mind that these conditions are constantly changing, due to the water currents that continually sweep past the pier at rates averaging from four to five miles per day. Consequently these conditions may vary considerably from day to day and even from hour to hour. The monthly means, however, will give a reasonably close approximation to the prevailing environments.

A comparison of the graphs in Figures 1 to 4 shows that the growth rates of the mussels have varied considerably, not only from year to year but also for the corresponding months of the years. The mean monthly increment in length for all groups was 3.43 mm. in the year 1940, 3.96 mm. in 1941, 5.43 mm. during the last seven months of 1942 and 5.11 mm. in 1943. It is evident from these figures that 1942 and 1943 were more favorable for rapid growth than either of the other two years and that 1940 was the least favorable. The lowest rate for any month of the four years was in August 1940. During that month the mean increment in length was only 1.6 mm., which was less than one-third as great as in the corresponding month of each of the three other years. The maximum rate occurred during April in 1940, during June in 1941, during July in 1942 and during May and July in 1943 (Figs. 1–4).

An examination of the environmental conditions, particularly as concerns temperature, storms and abundance of the phytoplankton during these years, will give some indication of the influence of each on the observed growth rates of the mussels.

First Year, 1940

The monthly growth rates of the mussels during this year were exceptional in that they showed fewer positive correlations with the temperature and with the

abundance of dinoflagellates than in any of the other years. Following a decrease in the rate during February there was a rapid increase to a maximum in May, fol-

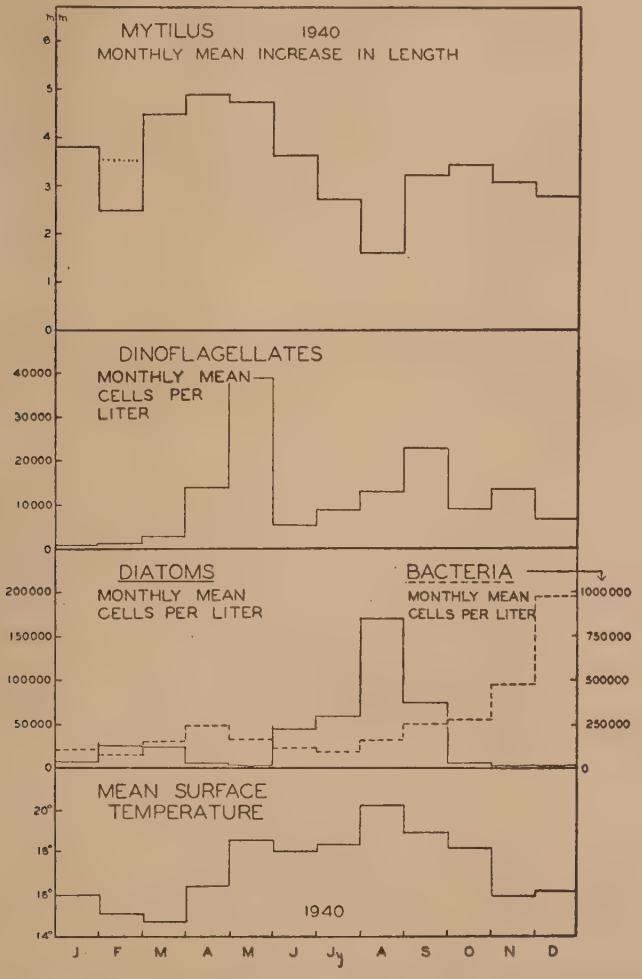

FIGURE 1. Graphs showing the average monthly growth rate of 453 mussels, divided into 11 groups according to age, and the abundance of dinoflagellates, diatoms and bacteria, as well as the average monthly temperature of the water during the year 1940. The depression of the growth rate in February was mainly due to a reduction of the feeding period to 22 days because of accidents caused by storms; the dotted line indicates the estimated increment if the accidents had not occurred. It was necessary also to estimate the growth in December because of an accident due to storm. The numbers of dinoflagellates shown in the graph differ in several cases from those indicated in Figure 4 of our previous paper (1942) because of erroneous data supplied to us at that time.

With some exceptions the growth rates were highest during those months having large dinoflagellate populations and in which the temperature exceeded 16° C.

lowed by a continuous decline to the lowest rate for any month of the four years in August (Fig. 1). The only explanation that can now be given for this exceptionally low rate in August is that for some unknown reason the organic detritus which furnishes the greater part of the mussel's nutrition was not present in sufficient quantity. The sharp drop in February was in part due to storms which necessitated removing the mussels from the sea and keeping them in the aquarium for six days. Since no increase in size occurs in the aquarium except when additional food is supplied (Coe and Fox, 1942), there was a possible feeding period of only 22 days during that month. Computed on the basis of the growth during that period, the estimated increase per day in February would be but slightly less than during the preceding month, as indicated by the dotted line in the graph (Fig. 1). A sharp rise in the growth rate in September and an additional increase in October was followed by the usual decline during the last two months of the year.

As a general rule, but with some conspicuous exceptions, the most rapid increment in size occurred during those months in which a large population of dinoflagellates was present and in which the temperature exceeded 16° C. Neither the diatoms nor the bacteria showed definite correlations with the growth rates of the mussels (Fig. 1).

Second Year, 1941

During the second year the growth rate was somewhat higher than in the preceding year, although the average number of dinoflagellates was smaller and the diatoms were less than half as abundant as in 1940. With the exception of February there was a continuous rise in the growth rate to a maximum in June, with a steady decrease thereafter (Fig. 2). The dinoflagellate population correspondingly reached a maximum in July, followed by a continuous decline to a minimum in December. Neither the diatoms nor the bacteria showed similar trends. The rate indicated for December is lower than it would have been except for a severe storm which allowed a feeding period of only 27 days.

Third Year, 1942

The experiment was interrupted for the first five months of 1942, but the last seven months of the year showed a greater increment in growth than in the corresponding months of any of the other years. The maximum rate occurred in July, followed by a continuous decrease during the rest of the year, with the exception of a slight rise in November, followed by a small decrease in December (Fig. 3). The water during those months contained an average of more than five times as many dinoflagellates as in the last seven months of the preceding year and the average monthly increment in the lengths of the mussels was 5.4 mm. as compared with 4.3 mm. in the corresponding period of 1941. The average number of diatoms was smaller than in any of the other three years.

Fourth Year, 1943

During the year 1943 the average monthly increment in size was considerably greater than in 1940 or 1941 but somewhat less than in 1942 (Fig. 4). By com-

paring the rate for December 1942 (Fig. 3), with that of January 1943, it will be seen that a sharp drop occurred during the latter month. The cause of this decrease in growth rate may have been due to a severe storm which necessitated transferring the experimental box from the sea to the aquarium, where it remained for four days.

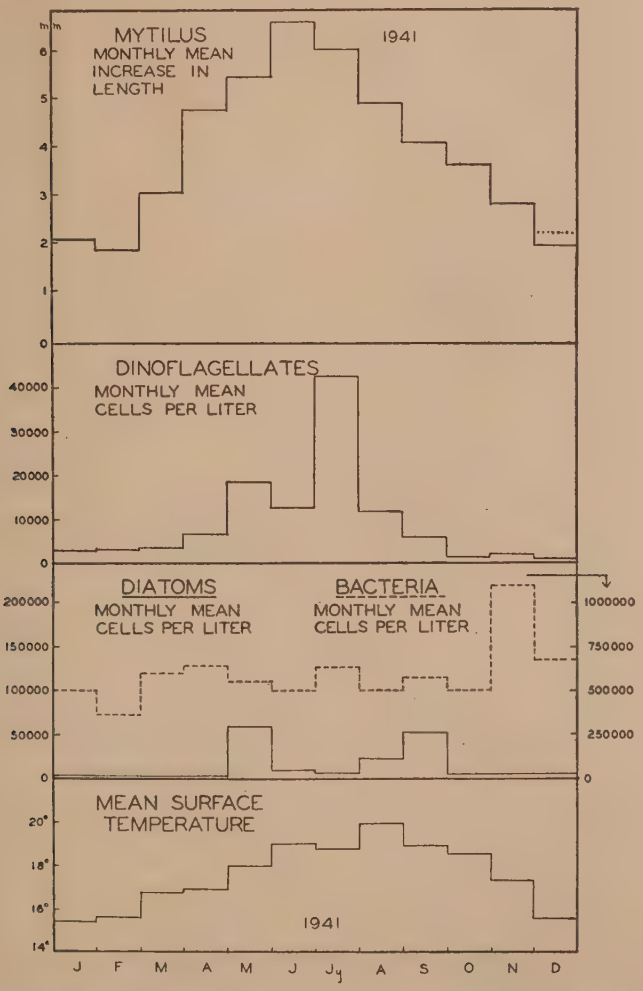

FIGURE 2. Graphs showing average monthly growth rate of mussels and abundance of dinoflagellates, diatoms and bacteria, as well as average monthly temperature of the water during the year 1941. The growth indicated for December represents a feeding period of only 27 days; the dotted line indicates the computed increase for a month of 31 days.

The most rapid increment in size occurred in those months having large populations of dinoflagellates, accompanied, presumably, by an abundance of organic detritus.

From this depression in January, the growth rate increased continuously until May, when the average increment was 6.4 mm. (Fig. 4). An unaccountable drop in the growth rate during June was followed by an average increment of 6.6 mm.

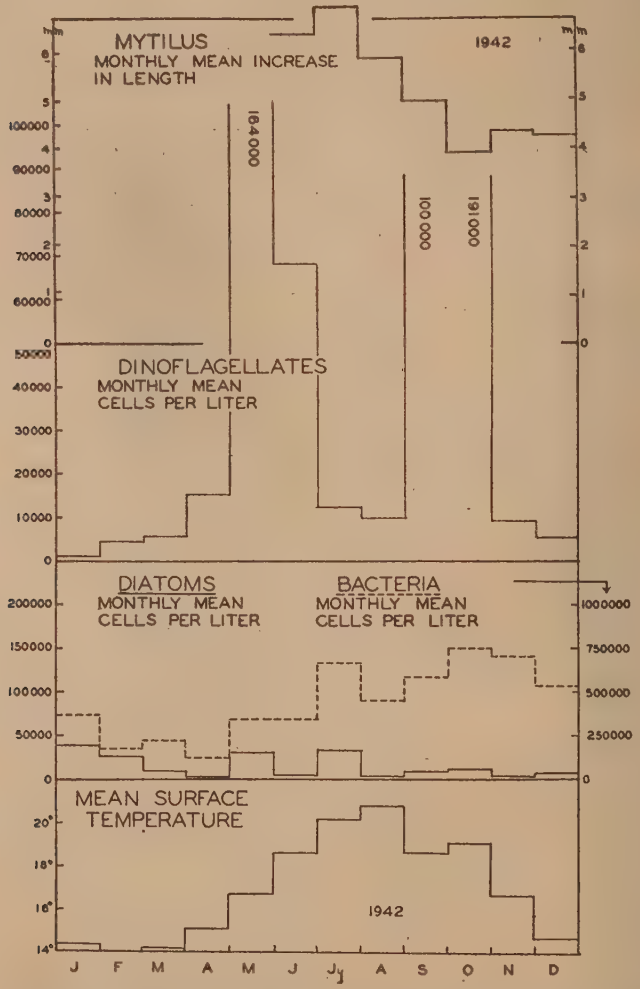

FIGURE 3. Graphs showing the mean monthly growth of mussels, the mean monthly temperature of the water and the mean monthly abundance of dinoflagellates, diatoms and bacteria during 1942.

in July, which was the highest rate for the year. Following the usual decrease in August, the rate continued high and steady during the two succeeding months; then, instead of the usual decline in November, there was a rise to an average of 6 mm., as compared with about 3 mm. in the corresponding month of 1940, 2 mm.

in 1941 and 4.3 mm. in 1942. In December the rate was but little more than half as great as during the preceding month (Fig. 4).

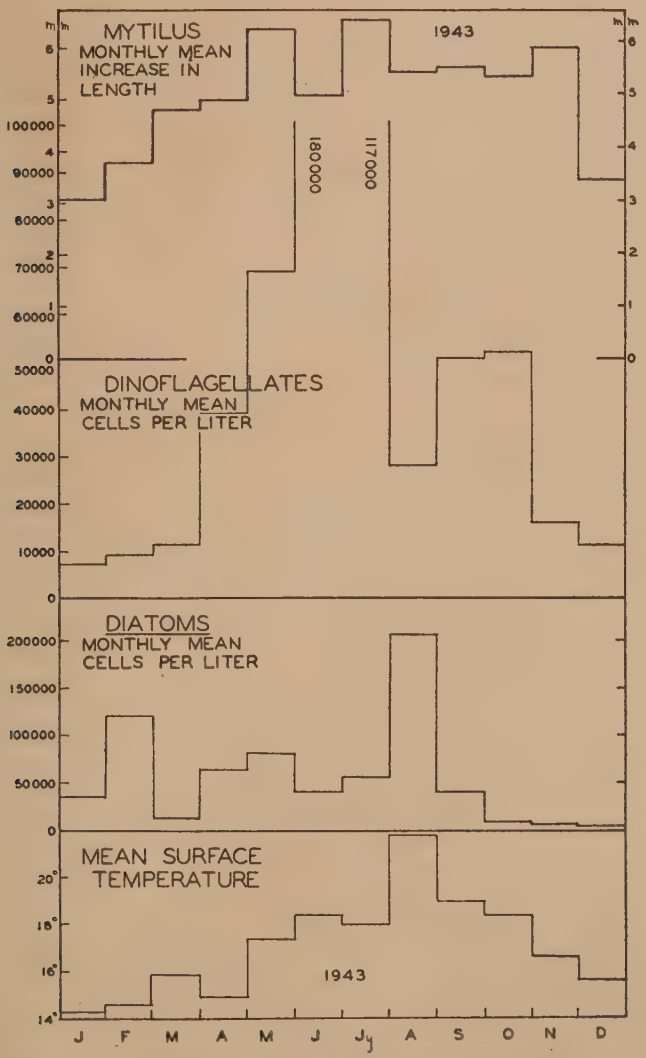

FIGURE 4. Graphs showing the mean monthly growth of mussels, the mean monthly temperature of the water and the mean monthly abundance of dinoflagellates and diatoms during the year 1943.

The average monthly increment in length was 5.1 mm. as compared with 3.43 mm. in 1940 and 3.96 mm. in 1941. This increased rate of growth was accompanied by populations of dinoflagellates more than four times as great as in either

1940 or 1941. The diatoms were also four times as numerous as in 1942, but again it should be emphasized that neither the dinoflagellates nor the diatoms furnish more than a small proportion of the food supply of the mussel.

Environmental Influences

The observations described on the foregoing pages show how great a variation was found in the growth rates of the mussels in different years and in different months of each year. The comparisons of these rates with the temperature and with the abundance of dinoflagellates, diatoms and bacteria are shown in Figures 1 to 4. There are obviously many other environmental conditions which are constantly exerting their influence on the growth of the mussels. Some of these may be of great importance but they are so sporadic in their action or so difficult to measure that no precise evaluation of their influence has as yet been possible.

There is little variation in salinity throughout the year and there is often a correlation between the amount of oxygen and the relative abundance of phytoplankton.

1. *Temperature*

As a general rule the rate of growth in mollusks increases with the temperature to a certain optimum and then rapidly decreases. Consequently the annual increment in length is greater in southern than in more northern localities because of the longer season favorable for rapid growth. The observations of Weymouth, McMillan and Rich (1931) on Siliqua, of Newcombe (1936) on Mya, of Chamberlain (1931) on Lampsilis, of Orton (1926–27) on Cardium and of Coe (1938) on Ostrea support this conclusion. The size eventually reached by the individual however is commonly much greater in the north because of the greater length of life.

At the pier of the Scripps Institution of Oceanography, where the experiment was conducted, the variation in the mean monthly surface temperature of the water during the year seldom exceeds 8° C. Both the low point of about 14° in winter and the high of about 22° in summer are well within range of the normal activities of the mussel. The highest temperature recorded at any time during these four years was 22.9° C., in August 1943, and the lowest was 13.4° in January 1943. Consequently growth continues throughout the year in this locality, although the rate of increment in length is only about half as great in midwinter as it usually is in the early summer. This decreased rate in winter is presumably due both to a lower state of metabolic activity and a decreased supply of nutritive materials.

In *M. californianus,* as in *M. edulis* (Loosanoff, 1942), feeding continues at temperatures both lower and higher than the extremes mentioned in the preceding paragraph. Under experimental conditions the California mussel will secrete byssus threads, ingest food and discharge feces at temperatures as high as 24 to 26° C. and to a less extent at 27 to 28°. The individuals subjected to the highest of these temperatures however died within 5 to 7 days. The lowest temperature at which ingestion and fecal discharge were found to occur was 7 to 8°. Since these mussels were subjected to the temperatures mentioned without any period of acclimatization from an aquarium temperature of 15°, it is considered probable that the figures given do not represent the extreme range of the mussels' potential metabolic activities.

Experiments previously made in this laboratory indicate that the maximum rate of filtration and maximum oxygen consumption take place at about 20° C., with distinctly lower rates below 15°. A decreased growth rate has occurred in August, the month of highest temperature, in each of the four years. But in 1940 the decrease began in June and reached its lowest rate in August, while in 1941 the highest growth rate occurred in June and in 1942 in July. In 1943 maxima occurred in May and July. The variability in the growth rates at corresponding temperatures in different years indicates that the food supply is more influential than small variations in temperature in determining the rates of growth. The prevalence of storms and high seas common in winter are doubtless detrimental to active growth.

2. *Food*

The mussel is essentially a scavenger, utilizing as food not only small unicellular organisms and dissociated cells but also the particulate disintegration products of any of the animals and plants which die in the vicinity or similar products which are brought from a distance by currents. Even the bacteria which cause the decomposition may themselves be utilized as an additional source of nourishment (Coe and Fox, 1942; Fox and Coe, 1943).

The constituents of the ingested materials have been ascertained at frequent intervals by examination of the stomach and intestinal contents and of the feces. The substances most commonly present are finely divided organic detritus, dinoflagellates, diatoms, silicoflagellates and bacteria; also tintinnids, flagellates, ciliates and other protozoans, as well as algal cells and fragments, algal spores, spermatozoa and ova (including those of its own species), together with inorganic substances such as particles of sand and shells. At times the organic materials may be ingested in amounts much greater than the mussels' capacity for assimilation. The excess, if not too great, may pass unchanged through the digestive system but in case of a very great surplus most of the material is rejected by the palps and is discharged from the mantle cavity as pseudofeces. No satisfactory evidence of selection, except as to size, from among these small cells and particles has been obtained, although chemically injurious substances are rejected, together with the larger cells and other objects.

Digestion in the mussel, with the exception of starch and glycogen, appears to be mainly or wholly intracellular. Many of the smallest objects and particles are phagocytized by the cells lining the digestive diverticula. Others are ingested by phagocytic cells which migrate into the lumens of the stomach and intestine and later return with their ingested materials through the epithelial lining of the digestive tract and thence to the connective tissues of the body, as Yonge (1926, 1931) has so fully described for the oyster. Most of the local dinoflagellates and many of the diatoms are far too large to be assimilated in this manner. Some of the starch and glycogen, on the contrary, undergoes extracellular digestion in the stomach through the action of enzymes in the style. No evidence of the digestion of cellulose, which forms the covering walls of most neritic dinoflagellates, nor of any cells with completely closed cellulose walls, has been obtained (Fox and Coe, 1943).

Diatoms. These organisms, either living or dead, furnish a small portion of

the mussels' nutrition. Their disintegration products are also utilized. They are usually present in numbers ranging from 1000 to 200,000 per liter but the large and spiny ones are not ingested. Many of those that enter the digestive tract are seized and digested by the phagocytic cells mentioned in a preceding paragraph, while others pass through the tract without apparent change.

The mean number of diatoms, as counted by the settling method, per liter of water for each month of the four years is shown in Figures 1 to 4 and the combined monthly averages for 1940, 1941 and 1943 in Figure 5. In none of these years has there been a direct correlation between the diatom populations and the mussels' growth rate, although positive correlations have been reported by Newcombe (1935) for Mya and by Nelson (1942) and others for oysters.

The number of diatoms in the water about the mussel beds has varied greatly from year to year. The average in 1940 was 38,700 per liter, in 1941 16,600, in 1942 12,600 and in 1943 54,300 (Figs. 1–4). The average monthly increase in the lengths of the mussels for the same years was 3.43 mm., 3.96 mm., 5.43 mm. and 5.10 mm., respectively. It is obvious that there was in these four years no direct correlation in the two groups of data. In spite of a four-fold increase in the number of diatoms in 1943 as compared with 1942 there was nevertheless a somewhat lower rate of growth in the mussels. This should not be surprising when it is realized that even if these organisms had been uniformly distributed in the water throughout the year, instead of occurring in dense swarms, the rate of filtration by the mussel is such that an adult animal could have secured no more than 200 million to 800 million per year. If all of these could have been fully utilized they would have furnished only a minute fraction of the material required for the upbuilding of the mussel's tissues and gametes. Such of these organisms as can be ingested by the mussel are so minute that it would require some 600 million to supply one gram of organic matter, while the adult mussel is estimated to need about 40 grams annually (Fox and Coe, 1943).

Bacteria. Bacteria are ingested in vast numbers (Figs. 1–3) but their total mass is so small that they have little quantitative influence on the mussels' nutrition (Fox and Coe, 1943).

Dinoflagellates. It has been mentioned that in each of the four years there was generally, but with some conspicuous exceptions, a rather close correspondence between the monthly and yearly growth rates of the mussels and the abundance or scarcity of dinoflagellates in the water. In 1940 the average daily number of these organisms per liter of water was 12,100, as compared with 9880 in 1941, 54,750 in 1942 and 49,500 in 1943. The corresponding average monthly growth rates of the mussels were 3.43 mm. in 1940, 3.96 mm. in 1941, 5.43 in 1942 and 5.10 in 1943. From these figures alone it may be concluded that the mussels grow most rapidly in those years in which the populations of dinoflagellates are the largest.

More precise evidence as to this association however is furnished by an inspection of the monthly data as shown in Figures 1–4. It has been emphasized in a foregoing paragraph however that a large proportion of the living dinoflagellates, which may be ingested in vast numbers, usually pass apparently unchanged through the intestinal tract and often constitute much of the fecal material. Their cellulose walls cannot be digested by the secretions in the stomach or intestine and there is no satisfactory evidence that they are phagocytized by the cells of the digestive diver-

ticula in any considerable numbers. Following the death of these unicellular organisms however, either before or after entering the mussel's digestive tract, they doubtless form particulate disintegration products which are readily assimilated. It is well known that species of Gonyaulax and less frequently of some other genera may be ingested in such numbers and the contained toxic substance accumulated in such quantity as to cause sickness or even death when the mussels are eaten by man.

It has been shown by Fox and Coe (1943) that the mussel filters the water at such a rate that the available supplies of dinoflagellates, even if they could be fully utilized, would furnish only a small fraction of the food which the mussels require for their growth and reproduction. Assuming a filtration rate of 2.5 liters per hour, or 22,000 liters per year, it would be necessary to have an average population of about 2000 of these cells per liter in order to supply one gram of organic matter in a year. This is only about two and one-half per cent of the amount which an adult mussel is estimated to require annually for the upbuilding of its tissues and gametes. During these four years the water has contained averages of 10,000 to 54,750 of these cells per liter but a large proportion of these were present in such dense swarms that relatively few of those that were drawn into the mantle cavities of the mussels could have been actually ingested; the others were presumably discharged as pseudofeces. Furthermore, as has been stated, many of those that are ingested usually pass through the digestive tract without visible change, while the large and spiny forms are seldom ingested.

Therefore any correlation between the abundance of dinoflagellates and the growth rates of the mussels must be merely indicative of other, associated sources of nutrition. The principal source is organic detritus.

Detritus. The organic detritus ingested by the mussel consists of various fragments of cells or of entire cells of minute size, as well as suspended proteins, lipids and polysaccharides. It may be recalled that the mussel obtains its food by secreting over its gills a thin sheet of mucus to which the particles are adsorbed. The mucus sheet with its attached particles is then drawn into the mouth. There is no evidence that the mussel is capable of securing substances in true solution until these have first been changed to particulate form through the agency of various unicellular organisms (Fox and Coe, 1943).

Consequently it may be concluded that, with the exception of refractory humus materials, cellulose, chitin and other indigestible substances, the total organic constituents of all marine organisms, from the smallest to the largest, are potential sources of nutrient for the mussel. After the death and disintegration of the animal or plant, the residual organic matter, or detritus, may remain suspended in the water for an indefinite period before it chances to enter the digestive system of the mussel. The amount available is obviously subject to great variation locally and it is to this variation that many of the differences in growth rates are ascribed.

Inspection of Figure 5, which indicates the combined average monthly growth rates for three years, will show that the rate increases from a low in January or February to a maximum in May, June and July. This period corresponds with the increasing reproduction of many of the invertebrates in the vicinity and elsewhere along the coast. The striped barnacle (*Balanus tintinnabulum*), for example, has minimum and maximum periods of reproduction coinciding almost precisely with the low and high growth rates of the mussels (Fig. 5). These reproductive peri-

odicities are undoubtedly associated with similar variations in the detritus which the barnacles yield, since a large proportion of the free-swimming larvae die without finding a place of attachment and presumably less than one per cent of those which succeed in transforming to the adult stage survive to reach sexual maturity

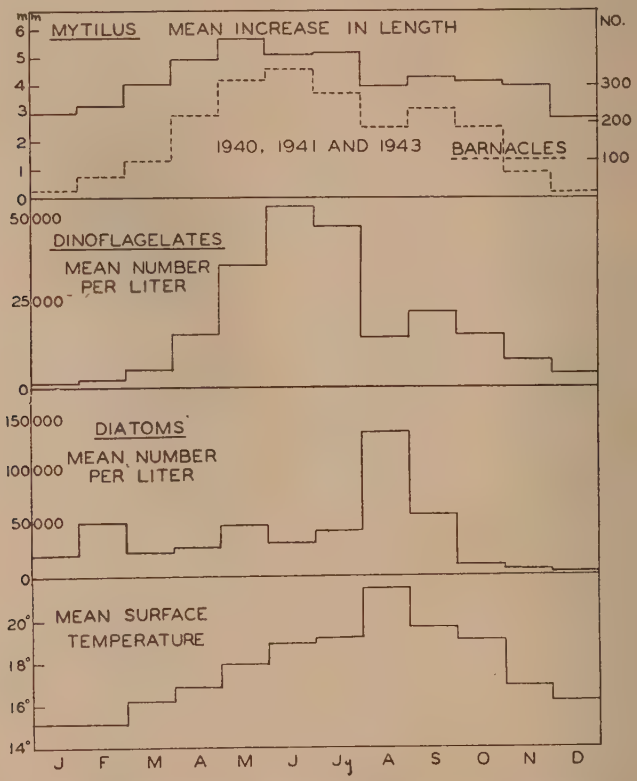

FIGURE 5. Correlations between the mean monthly increment in length of the experimental groups of mussels for the years 1940, 1941 and 1943, the mean numbers of dinoflagellates and diatoms per liter and the mean temperature of the water. The year 1942 is not included because of lack of complete data for the first five months of that year. Except for the decrease both in growth rate of the mussels and numbers of dinoflagellates during August, when the temperatures were highest, the general correspondence of three of these groups of data is evident. But this correspondence does not hold for the diatoms.

As indicative of the relative amount of organic detritus presumably available in each month, the estimated average numbers of barnacles which became attached to each square inch of surface of submerged plates in previous years have been included. These numbers indicate a close correlation with the growth rates of the mussels.

(Coe, 1932). The disintegrated bodies of those that perish doubtless supply more nutritive material to the mussel than can be obtained from the living phytoplankton.

In some years the growth rate has been more or less distinctly bimodal, rising to a maximum in late spring or early summer, followed by an invariable decrease in

August and in three of the four years with another rise preceding the decrease at the end of the year. In three of the four years the dinoflagellates showed somewhat similar bimodal periodicities, with distinct spring and autumn maxima, and this has been shown to be the average condition of these organisms for the twenty preceding years (Allen, 1941). The diatom populations, on the contrary, reached a conspicuous maximum in August in 1940 and in 1943, although in many other years that has been a month of extremely low production.

The bimodal periodicities in the growth rates of the mussel are closely parallel with similar periodicities in the reproduction of many of the associated invertebrates, including the mussels themselves. The growth rates of the entire mussel population would obviously be complicated by the reproductive processes but these complications were avoided in this experiment by using sexually immature individuals.

It is unfortunate that no precise measurements are available relative to the local variations in the amount of organic detritus, but the seasonal differences in the rates of growth of the mussels and other detritus feeders presumably afford a fairly reliable criterion.

Summary

This study offers additional evidence as to the complexity of the environmental conditions found along the shores of the ocean and which affect so profoundly the lives of the organisms residing there. Variations in the growth rates will obviously depend upon the interaction of several of these conditions, not the least important of which are the temperature and the character and abundance of the food supply.

Furthermore these environmental conditions are constantly changing, due in part to the continual motion of the water. At the locality where the foregoing observations were made, there are not only the variable currents caused by wind and tidal changes, but there is also a drift along the coast at a rate averaging four to five miles per day. Consequently the water in which the mussels are living and the conditions associated therewith may differ not only from month to month but also from day to day and even from hour to hour. In one week there may be ten to fifty times as much phytoplankton in the water as in the following week. The yearly averages are more stable but these may vary by more than five fold.

Monthly correlations, extending over four years, between the growth rates of the mussels and the prevalent environmental conditions offer conclusive evidence that the most rapid increase in size takes place at temperatures from 17 to 20° C., although growth continues less rapidly at 14° or lower. Feeding continues at a temperature as low as 7 to 8° and as high as 27 to 28°.

The average number of diatoms per liter was 38,700 in 1940, 16,600 in 1941, 12,600 in 1942 and 54,300 in 1943. The average number of dinoflagellates for the same years was 12,100, 9880, 54,750 and 49,500, respectively. The average monthly increment in the lengths of the mussels was 3.43 mm. in 1940, 3.96 in 1941, 5.43 mm. in 1942 and 5.11 mm. in 1943, indicating a yearly variation of more than 50 per cent.

It is obvious that the two years with the largest dinoflagellate populations have been conducive to the most rapid growth of the mussels, but an increase of four fold in abundance has been associated with an increased growth rate of only 42 per cent. The correlation noted is evidently not direct, since the living dinoflagellates

can supply only a small fraction of the mussels' nutritive requirements. Both mussels and dinoflagellates appear to thrive under the same environmental conditions. That the living diatoms and bacteria are of even less importance in the life of the mussel is indicated not only by the small amount of organic matter that they contain but also by the fact that the mussels grew most rapidly in the year with the smallest number of diatoms and least rapidly in the year when the number was three times as great.

More direct correlations with the growth rates of the mussels are found in the reproductive periodicities of various invertebrate populations which contribute so largely to the available organic detritus and thereby to the mussels' nutrition.

The principal food supply of this species of mussel consists of minute particles of organic detritus derived from the disintegration of the cells of all kinds of marine organisms, both animals and plants, supplemented by living and dead unicellular organisms of minute size as well as living and dead gametes. There is no evidence that organic matter in true solution can be utilized until after it has been changed into particulate form by the action of unicellular organisms.

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TUDIES OF THE VITALLY STAINED GOLGI APPARATUS

III. THE METHYLENE BLUE TECHNIQUE AND SOME OF ITS IMPLICATIONS

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ONE TEXT FIGURE AND FOUR PLATES (TWENTY-SEVEN FIGURES)

INTRODUCTION

In the first study of this series (Worley and Worley, '43) the Golgi odies in the developing embryo of the tectibranch mollusc Navanax vere revealed clearly and unmistakably by allowing the eggs to de- elop in a very dilute concentration of methylene blue in sea water. The methylene blue stained the chromophilic components of the Golgi odies with the result that they appeared very dark blue against a ractically colorless background. The Golgi body chromophobes re- nained unstained so that a sharp contrast between chromophiles and hromophobes was obtained. It was also found that the classical gas- ropod Golgi configuration produced by the osmic methods could be duplicated by shrinking, through desiccation, the vitally stained cells.

The ability of the gastropod Golgi apparatus to be stained with ertain vital dyes has long been recognized. When Bowen ('28) re- viewed the various observations he obviously was of the opinion that he pulmonate Golgi apparatus was exceptional in this regard. On his point, he says (p. 308), "As in the case of intra-vitam observations vithout staining, so in the case of vital dyes the outstanding example of success is the male germinal tissue of the pulmonate molluscs. Ap- arently the same characteristics which make the Golgi apparatus in his case so amenable to staining methods of all kinds and to vital obser- vation also lend themselves readily to vital staining."

Since the time of Bowen, however, the vital dyes, particularly neutral ed, frequently have been successfully used in demonstrating indivi- dual Golgi bodies in a wide variety of species. Hirsch ('39) has con- tributed much to our knowledge of this phase of cytology and has

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furnished a very thorough treatment of the literature on this subject. But few, if any, investigators have succeeded in staining vitally the classical Golgi "network" and students of the vital staining methods are fairly unanimous in denying the existence of such a structure in the living cell. However, they have never succeeded in providing an unequivocal proof for this assertion, nor have they gained universal acceptance for their views. Quite the contrary, those who defend the "network theory" are often outspokenly critical of the vital methods and there seems to be no question but that at least certain of the vital dyes are quite unreliable and may even lead to the formation of artifacts in the cell. Some of the "Krinome" of Chlopin ('27) may be an example. Thus, doubt is frequently expressed in the literature regarding the validity of considering any of the vitally stained cytoplasmic inclusions identical with the elements revealed by the osmic and silver methods.

The present study is concerned with determining to what extent methylene blue can serve generally as a vital stain for the Golgi apparatus. This stain has seldom been used for this purpose in the past, except in connection with the germinal tissue of molluscs. Ludford ('35) obtained what was interpreted as a negative image of the apparatus in fibroblasts stained with methylene blue. It is also the purpose of this study to ascertain whether or not, in general, the classical Golgi configuration is the result of distortion of the living Golgi elements during fixation. Since the paper deals to a considerable extent with technique, it has been found desirable to describe the methods in connection with the description of results on each of the tissues studied, rather than collectively under a separate heading.

This work was carried out at the Scripps Institution of Oceanography. I am indebted to the Director of the Institution and to the Regents of the University of California for providing space and facilities. I am very grateful to Prof. Wesley R. Coe for his kind advice and criticism and for the willingness with which he has always been ready to inspect various preparations.

MOLLUSCS

Since the vital dyes have a reputation for producing "neoformations" in the cell, considerable attention has been given to this problem in my work with methylene blue. Specifically, for some time concern was felt lest the structures being identified as Golgi body chromophiles in the molluscs, were in reality merely dye droplets formed

the surfaces of contact between certain yolk spheres (identified as olgi body chromophobes) and the ground cytoplasm. This never emed to be a very likely possibility, however, because the methylene ue was always deposited in living preparations of marine eggs in e same relation to the chromophobic matter as was the reduced smic acid in the fixed material. Therefore, had the methylene blue eposits proved to be "neoformations," the osmic acid configuration ould likewise have had to be considered artificial and the reality of olgi body chromophiles in general would have been open to question. All doubts on this important point are removed, however, when nstained gastropod embryos are crushed so that the Golgi bodies pread out onto the slide and can be examined individually. Such in-usions are of the same form as those seen in stained eggs and chromo-niles and chromophobes can usually be differentiated. Some of these olated, unstained Golgi bodies are reproduced in figure 3 for com-arison with the stained elements (in vivo) of figure 2. Lines have een drawn connecting stained and unstained Golgi bodies thought to be a similar state of development. Since chromophiles can be distin-uished from chromophobes in the unstained inclusions, the chromo-niles cannot possibly be considered "excretion droplets" formed in sponse to the presence of the vital dye and, at the same time, it is pparent that osmic acid impregnates a preformed structure. It is ow known, however, that the clear demonstration of the differentia-on between chromophile and chromophobe in the large Golgi bodies the molluscs is facilitated, in part, by the fact that under slight ressure, such as is caused by the cover glass, the chromophile has a ndency to "slip" to either side of the chromophobe thereby revealing e internal structure of the Golgi body. This is not surprising in ew of the very fluid nature of the chromophilic Golgi substance.

Macdougald ('37) states (p. 41), with regard to neutral red, that staining did not seem to be affected by concentration, except that ronger solutions gave quicker results." This statement is more less applicable to methylene blue, but heavy concentrations appear produce thicker chromophiles just as osmic acid may result in either light or a heavy impregnation depending on the length of time is allowed to act. This tendency of the chromophile to absorb stain as led to the use of the term "segregation apparatus" for the Golgi odies of some tissues. In most cases the two terms are probably nonymous, a conclusion reached as long ago as 1928 by Dawson for e amphibian erythrocyte.

ARTHROPODS

Malpighian tubules of insects

Observations have been made on the malpighian tubules of several species of grasshoppers of the genera Hippiscus and Melanoplus. To stain the Golgi inclusions, the posterior tip of the grasshopper abdomen was snipped off with scissors and then, by pulling on the head, the whole digestive tract with the attached tubules was removed from the chitinous exoskeleton. Digestive tube and tubules were dropped into Ringer's solution containing 1:500,000 methylene blue. The Golgi bodies in the tubules take up the stain within an hour and the stain is usually localized within them. A diffuse staining of the nuclei and especially of the cytoplasm should be avoided, although a faint staining of the nucleolus is not considered undesirable. Sometimes poorly stained tubules occur side by side with well stained ones in the same material. The inclusions are readily seen under lower powers (fig. 4) while, at higher magnifications (fig. 5), they appear to be approximately spherical vesicles. A distinction between chromophiles and chromophobes cannot be observed. A good osmicated preparation from the same species (fig. 6) has been included for comparison. In this case, a reasonable correspondence between the fixed and the living inclusions has been obtained, but it should be noted (by comparison with fig. 5) that there has been no noticeable fixation shrinkage.

The inclusions in neither the fixed nor the living material bear much resemblance to the classical arthropod Golgi elements. There are no rods or loops, nor definite rings or crescents, but in the fixed tubule chromophiles can sometimes be distinguished from chromophobes (arrows). It is possible that one would not identify these inclusions as Golgi elements at all if he failed to realize that the simple vesicle is one of the commonest Golgi-body types. Because of the resemblance between these bodies and some of the Golgi elements in molluscs, however, and because of their reaction to osmic acid with the differentiation into chromophile and chromophobe, these are almost without question the Golgi structures. Furthermore, when the freshly stained material is allowed to undergo desiccation on the slide (thus simulating fixation shrinkage) the chromophiles become more or less rod-shaped and consequently present the classical picture.

Salivary glands of the Chironomus larva

The salivary glands of the midge larva have been favorite objects of investigation for many years and there has been a conflict in opinion

regarding the cytology of these cells. Parat and Painlévé ('24) claimed that the only important cytoplasmic elements were the "chondriome" (mitochondria) and the "vacuome" (inclusions in the cell stainable in life with neutral red). The presence in the living cell of a classical Golgi apparatus was denied. Krjukowa ('29) and Beams and Goldsmith ('30) demonstrated, in fresh material, neutral red bodies and, in fixed cells in addition to mitochondria, a variety of typical Golgi rings, crescents, loops and rods. Gatenby ('32) obtained similar results, but he definitely regarded the neutral red inclusions as artifacts. He says (p. 261), " . . . When one speaks of the 'vacuome' in connection with Chironomus salivary cells, one is not referring to preexisting structures, but to the faculty that the ground cytoplasm has of segregating a dense concentration of dye into discrete globules. The latter may later become connected by anastomoses as the toxic effect increases." Later, Borghese ('35) was unable to demonstrate either "vacuome" or Golgi inclusions. To date no one appears to have been successful in reconciling these various views.

To demonstrate the Golgi inclusions with methylene blue, Chironomus larvae were placed in finger bowls of pond water along with the case-composing debris that surrounded the larvae when collected. Methylene blue sufficient to bring the concentration to about 1:20,000 was introduced into the pond water, but adsorption of so much of the dye by the dead organic matter resulted that more methylene blue had to be added repeatedly. The larvae were examined after 24, 48 and 72 hours, but in all cases the salivary gland Golgi bodies so revealed showed a similar structure, viz., they were in the form of spherical vesicles of varying size fairly evenly distributed throughout the cytoplasm (fig. 9). No internal differentiation within the vesicles was discernible. In cells believed because of the abundance of secretory products to be synthetically more active, the Golgi bodies averaged larger, but again were without apparent internal differentiation (fig. 11). A close topographical relation between the vesicles and the unstained secretion droplets was frequently noted (fig. 11), but no definite indication as to the method whereby such products are elaborated in these cells has thus far been secured.

These vesicles (in all probability, in this instance, the "vacuome" of Parat and Painlévé) are unquestionably the living representative of the Golgi system in these cells since they closely resemble certain of the Golgi elements of the molluscs and show a similar reaction to methylene blue. What, from the standpoint of the reader, may be more important is the fact that the classical Golgi apparatus described by

Krjukowa, Beams and Goldsmith, and Gatenby, results from the collapse of these vesicles during the process of fixation. This can be proven by treating the living, methylene blue stained material with a drop of hypertonic salt solution (sea water, 3.5% salinity, was used) thus simulating fixation shrinkage. Better still, salt solution can be allowed to seep under the cover slip while the same cell is continually kept under observation. As a result of this treatment, the vesicles of the relatively inactive cells collapse and form the rings, rods, loops and crescents characteristic of the classical picture (fig. 10). This is a constant and very striking result.

Many of the bodies now appear as chromophilic rings each with a more or less rounded internal tubercle or thickening at one point. In this condition they somewhat resemble the living, simple vesicular Golgi bodies of molluscs and the conclusion is pertinent that these vesicles in the living Chironomus are very similar to those of molluscs except that the chromophilic pellicle when impregnated with methylene blue is not sufficiently transparent to enable one to distinguish the internal structure of the vesicle.

When the active gland is similarly treated with hypertonic salt solution, a variety of figures results (fig. 12). Rings and crescents are formed, but in addition, there appear elongated coarse threads which somewhat resemble certain familiar punctuation marks, such as exclamation points and large-headed commas. Many of these figures are relatively broader at one end and thus appear somewhat wedge-shaped. The form of these artifacts is of some importance in connection with observations to be reported later. They fall far short of representing faithfully the Golgi system of the living cell, but it is apparent that they are of some value in elucidating the internal structure of the vesicles.

It is worth emphasizing that figures 10 and 12 showing the classical Golgi apparatus are photographs each taken within 10 minutes after the removal of the tissue from the body of the living larva. In the past, it has usually been considered a foregone conclusion that to obtain such images required anywhere from 3 to 8 days of osmication. It should also be pointed out that figures 10 and 12 are photographs of the same cells as are illustrated respectively in figures 9 and 11 and that less than 5 minutes elapsed between the taking of corresponding photographs.

It now seems probable that the anastomosing filaments reported by Parat and Painlévé and by Gatenby in vitally stained material were the result of the shrinkage or partial desiccation of the material while

under observation. It is also evident that the Golgi elements described by Krjukowa, Beams and Goldsmith, and Gatenby represented artificial forms of the apparatus, but that a well developed Golgi system exists in these cells in life, contrary to the conclusion of Borghese, there can be no doubt.

Embryos of the sand crab, Emerita analoga (Stimpson)

Eggs and embryos of the sand crab have been developed in 1:500,000 methylene blue in sea water. The tough egg membranes prevented complete staining with methylene blue for 48 to 72 hours, but at the end of such periods many of the embryos appeared to be in good condition and the stain was localized in what are believed to be the Golgi bodies.

In the molluscs, it was found that the embryonic yolk spheres typically were elaborated within Golgi bodies through a process of intrasomal action, and later discharged. The active Golgi elements, therefore, had to be of sufficient size to accommodate the developing spheres of yolk. The interesting thing about the sand crab embryo is that some yolk spheres are particularly large and the Golgi bodies that elaborate them are, as would be expected, correspondingly so. Observations on the structure of the apparatus are consequently facilitated. However, studies of these giant forms (fig. 7) only helps make more clear what has been determined in other species — both chromophile and chromophobe each appear homogeneous and the chromophile can readily be seen to enclose completely the latter. This is in agreement with the view of Hirsch ('39). The resemblance between the Emerita Golgi body (fig. 7) and some of those in molluscs (figs. 2–3, line C) should be noted. In molluscs, the smaller of the two chromophobes in bipolar bodies of this type is frequently largely lipoidal in character, while the large chromophobe is primarily protein.

ECHINODERMS AND ANNELIDS

Echinoderm eggs of all the immediately available species have been cultivated in methylene blue, but none was found that could be reared successfully even in very dilute solutions of this stain. The species examined were the echinoids Dendraster excentricus (Eschscholtz), Strongylocentrotus purpuratus (Stimpson), Lytechinus pictus (Verrill) and the starfish Pisaster ochraceus ochraceus (Brandt). In eggs of all of these species, brought gradually into 1:200,000 methylene blue from 1:1,000,000, abnormal development becomes evident usually before the completion of gastrulation.

In the early stages, when development appears normal, the Golgi inclusions readily stain with methylene blue. That it is the Golgi bodies which take up the dye has been determined by comparing their structure in vitally stained material with their condition in Kolatchev preparations of eggs of the Atlantic sea urchin, Arbacia punctulata (Lamarck). In all of these species, the Golgi bodies are in the form of homogeneous granules and droplets and spherical vesicles, the latter showing distinct chromophobes in contrast with the chromophiles. Simple vesicular forms are particularly abundant in the gastrula (fig. 8) and are then larger than in earlier stages. This is evident in the osmicated, as well as in the living, material. Puddingstone-like Golgi masses, elaborating droplets of fat (?) have been observed in the larvae, but since the embryos otherwise were abnormal in shape and size, we cannot conclude that this is the normal period for such activity. It should be noted, however, that the larval spicules were observed to originate completely unassociated with the Golgi elements.

Fixed and sectioned echinoderm eggs are useful in showing the localization of the Golgi substance within the individual cells and, after gastrulation, make it possible to compare the position of the apparatus in the archenteron cells with that in the "ectoderm." The fixed material reveals many small "crescents" in the cleavage stages which, in the blastula, enlarge and migrate to the outer cytoplasm beyond the nuclei, adjacent to the surface of the embryos. Gastrulation consequently localizes those of the young gut cells adjacent to the lumen. By this stage, the Golgi bodies in the fixed material are V-shaped or rod-shaped, but it is clear that this is not the form in which they occur in life. Kolatchev fixed and osmicated eggs showed a 20% shrinkage which practically obliterated the blastocoel in the blastula stages.

The only annelid egg studied vitally was that of the lugworm, Arenicola claparedii Levinson. The small, droplet-like Golgi bodies in this egg are pigmented in life and can easily be seen in the living egg. They also readily absorb methylene blue (fig. 13) and blacken with osmic acid (fig. 14). They are quite resistant to fixation and give the impression of being more or less solid. They appear to consist entirely of chromophilic material and even in the fixed preparations a differentiation into chromophile and chromophobe cannot be observed. The failure of these bodies to collapse readily is in keeping with the impression gained throughout this work that Golgi droplets are usually relatively solid in comparison with the more differentiated types, having what appears to be the consistency of a gel. It would certainly seem that the assumption of a spherical form by a substance as variable in

shape as the Golgi material in developing eggs, could be taken as an indication of fluidity and this is often, but certainly not always, the case. It should be continually kept in mind, however, that a homogeneous appearance of a Golgi body does not necessarily mean a homogeneous structure. Little indication of activity has been observed in the minute (about 1 μ) Golgi bodies of the Arenicola egg during the first 14 days of development. This lack of activity is correlated both with their homogeneous structure and solid character which in all species thus far examined have been indicative of a synthetically inactive condition. This conclusion coincides with that of Hirsch ('39) who includes Golgi elements of this type under the term "presubstance."

AMPHIBIAN AND MAMMALIAN SMOOTH MUSCLE

Tadpoles of Hyla regilla Baird and Girard, nearing metamorphosis, were kept in 1:20,000 methylene blue in pond water and examined at intervals up to 8 days. The adsorption of much of the stain by dead organic matter made a continual addition of methylene blue necessary and the concentration varied accordingly. Many of the internal organs of the tadpole, particularly the pancreas, liver, gall bladder and gut were well stained after 24 hours.

The intestinal smooth muscle cells invariably react to staining in a characteristic manner and show a variable number of homogeneous-appearing, spherical vesicles of different sizes (fig. 15). It is very unlikely that these are wholly artificial structures since stained animals frequently live and feed indefinitely and even undergo metamorphosis. Furthermore, when this tissue is treated with hypertonic salt solution, these vesicles collapse and due to their soft gel consistency they form in each cell a chromophilic fixture that closely resembles and is without much question identical with the classical lamellar Golgi apparatus of the fixed amphibian smooth muscle cell (figs. 16 and 17).

Similar results can be obtained in mammalian tissue by the same methods except that the methylene blue is administered intra-vitam. Kittens about 2 weeks old have been used. Eighteen hours before the tissue is desired for study, 10 cc. of 1:400 methylene blue in physiological salt solution and warmed to body temperature is injected intraperitoneally, followed by a similar injection 6 to 8 hours later. At the end of the 18-hour period, various internal organs are found to have accumulated the dye. Smooth muscle of the intestine and bladder is usually well stained, as well as many other tissues and organs, including the pancreas, liver, kidney cortex, spleen, thymus and occasionally,

the pituitary and thyroid. The observations on smooth muscle confirm those described for this tissue in the tadpole.

THE AMPHIBIAN TONGUE

The tongue of the adult frog is a particularly suitable organ to use for the demonstration with methylene blue of both the living Golgi system and the classical Golgi configuration. A freshly excised tongue is soaked for from 3 to 4 hours in 1 : 100,000 methylene blue in Ringer's solution. When ready, small pieces are cut from the blue periphery of the organ and then left for a few minutes in hypertonic salt solution (about 3 or 4%). A piece is then placed on a slide and a cover slip is pressed down very firmly so that epithelial cells are freed and float out into the fluid. Such preparations usually reveal the Golgi picture characteristic of these cells in osmic preparations (figs. 18–20). The living Golgi elements of these cells can be observed by omitting the salt water treatment and taking care not to crush the material (fig. 28).

THE AMPHIBIAN PANCREAS

The stained frog tadpole pancreas has proved to be of considerable interest. Stained tissue teased apart in Ringer's solution reveals thousands of zymogen granules, appearing pale green, which have been dislodged from the ruptured cells. The methylene blue bodies consist of numerous dark blue granules (fig. 1, A), homogeneous appearing droplets or vesicles (fig. 1, B) and numerous large, more or less spherical, pale blue puddingstone-like masses, containing paler vesicles (fig. 1, D). The paleness of these large forms has made adequate photographs impossible.

When one of these large masses is studied immediately after teasing or crushing, the discharge of one or more vesicles as solid pale green granules, which look exactly like the already present zymogen granules, can often be noted. Osmic acid applied to the fresh mount does not appear to blacken these granules, so they probably cannot be considered fat and it is thought that this constitutes an observation of the discharge of zymogen granules from the active amphibian apparatus. It is believed that this observation helps confirm, on living material, Bowen's ('26 b) view of the function of the Golgi apparatus in sero-zymogenic cells. Certainly the Golgi apparatus plays a major role in this process, but these statements are not intended to imply that it is the only constituent involved. These compound vesicular forms are frequently seen in osmic preparations of amphibian exo-

crine glands, but it is doubtful if they have ever, heretofore, been considered Golgi elements, partly, perhaps, because they are not always blackened. There seems frequently to be a tendency on the part of actively secreting Golgi bodies to lose their affinity for osmic acid. Pollister ('39) has remarked on this point with reference to frog pancreas cells. This is correlated with the failure of the hypertrophied apparatus to stain deeply with methylene blue and may be coincident with a decline in its ability to absorb substances from the cytoplasm as the apparatus turns its attention entirely to the discharge of the elaborated products.

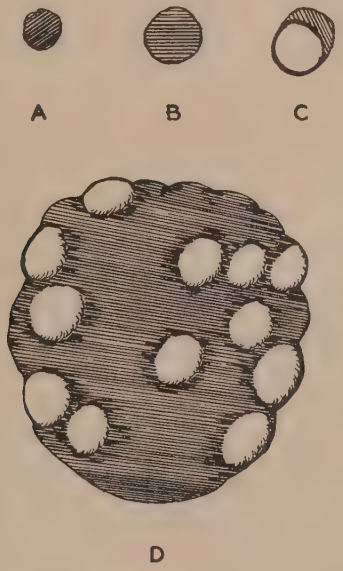

Fig. 1 Types of living Golgi inclusions found in the acinar cells of the frog tadpole pancreas. A, granule. B, droplet or vesicle, probably the latter. C, vesicular form revealed by treating the material with hypertonic salt solution. D, puddingstone-like form interpreted as zymogenic.

On the other hand, observations on molluscs have indicated that the Golgi apparatus has a tendency to be less chromophilic when involved in protein elaboration than when engaged in the formation of fat. Some of the observations I have made on the readiness with which the osmicated apparatus becomes bleached out with very dilute hydrogen peroxide in comparison with the speed of bleaching of fat drops, indicate that at one time the Golgi substance is much more lipoidal and at another relatively more protein in character. More specifically, in some instances a Golgi body 2 μ in diameter will bleach out more rap-

idly than a 6 μ fat droplet, but less readily than a 2 μ fat droplet. In other cases, small Golgi bodies, presumably relatively more protein in composition, resist bleaching much longer than the largest fat droplets, as is well known. These observations seem to bear out the idea of a fluctuating chemical composition which probably can be correlated with the nature of the available cellular raw materials. Thus the active amphibian Golgi substance of the pancreas, charged as it may be with protein precursors awaiting elaboration, may possibly be thought of as being diluted (?) by these raw materials and, for this reason, may perhaps be less chromophilic.

Fresh preparations of the tadpole pancreas, treated with salt solution, frequently show simple vesicular Golgi bodies with clearly differentiated chromophiles and chromophobes (fig. 1, C). Fixing fluids also often contract the chromophile into a rod, crescent or ball, leaving a partly naked chromophobe.

THE MAMMALIAN PANCREAS

The pancreas is one of the first organs in the kitten to absorb methylene blue after injection. The effects of such treatment on the alveolar cells are well known, but not completely understood. One group of workers, notably Beams ('30), Ludford ('30), Gatenby ('31) and Chang ('35), are of the opinion that the intensely stained bodies that appear in such cells are newly formed dye droplets lacking a counterpart in the untreated cell. Bensley ('11), Covell ('28), Ma ('30), Ichinomiya ('31), Hirsch ('39) and others believe that these inclusions are preformed structures and that they constitute to a greater or lesser extent the Golgi apparatus of the living cell. Crucial proof of neither of these views has been furnished.

In the cat pancreas these so-called "dye droplets" are nearly spherical bodies, showing some variation in size, especially in different cells. They are very similar to those visible in the frog tongue as a result of similar treatment (fig. 28) and their resemblance to the Golgi vesicles of many invertebrates is noticeable. They stain very dark blue and stand out distinctly from the surrounding cytoplasm. The zymogen granules do not stain. No differentiation into chromophile and chromophobe can be noted, but that we are dealing with a fluid vesicle and not a more or less solid granule or gel-like droplet is apparent when hypertonic salt solution is applied. Under such circumstances the vesicles react in either of two different ways, correlated apparently with their size. The smaller vesicles transform into rings about 1 μ in diameter. These show local thickenings like those resulting from

similar treatment of the relatively inactive cells of Chironomus and they also resemble the tiny rings frequently seen in osmicated sections of the pancreas (fig. 22, arrow). In the majority of cells, the larger vesicles elongate and form coarse threads between the zymogen granules (similar to those formed in the kidney as the result of such treatment, fig. 21). These threads are often larger at one end and they may be bent or curved. Again, as in the relatively active cells of Chironomus, exclamation marks and comma-like forms are in evidence. The resemblance between such artificially produced forms and the structures seen in osmic preparations, strongly suggests their identity. Frequently both threads and vesicles are present in the same cell.

An even more striking effect occurs as a result of treating relatively active, vitally-stained cells with Kolatchev fixing fluid. The best results are obtained by dropping small pieces of vitally stained tissue into the fixative, after a few minutes teasing on a slide and then allowing the material to dry for half an hour or more under a cover slip. This treatment produces a great variety of figures (figs. 23–27) which are with very little doubt identical with the classical Golgi "networks" described for these and similar cells.

It should be borne in mind that the figures are photographs of these objects as they are seen in total mount and consequently they appear somewhat more elaborate than when viewed in thin sections. In such preparations the Golgi configuration naturally appears dark blue since it is the result of the distortion of the vitally stained cytoplasmic inclusions. When light is transmitted through a yellow filter these figures appear black on a yellow background just as they do in the familiar osmic preparations. For this reason, the use of a filter assists the observer in appreciating the identity of these structures. The technique just described has been used successfully on the thyroid, pituitary, cardiac muscle, bladder, adipose and other connective tissues of the cat and the guinea pig, in addition to those already mentioned.

DISCUSSION

These observations indicate several things quite clearly. First, if the deeply-stained bodies in the pancreas were merely dye droplets, as so many authors have maintained, they could not possibly be demonstrated in osmic preparations that have never been subjected to vital dye treatment. Second, if they were the result of the vital stain, the collapse of the smaller ones would certainly not reveal an internal structure in the form of the small chromophilic knobs. Third, that

appropriate treatment of the methylene blue stained bodies would invariably result in the classical Golgi picture, characteristic of each particular cell-type, unless the two are identical is out of the question. Such a coincidence might not be surprising in one or two instances, but when the well-known Golgi configuration can be duplicated by the same method in such diverse cells as those of the Chironomus salivary gland, those of amphibian smooth muscle and those of the cat kidney, pancreas, thyroid and pituitary, as well as the molluscan embryo, the correspondence is certainly a real one and any other explanation is not readily apparent.

The fact that both coarse threads and unaltered Golgi vesicles may sometimes be present in the same cell, is probably responsible, in part, for the conclusion reached by some authors that "dye droplets" (or "vacuome"?) and Golgi apparatus can be demonstrated in the same cell and are, therefore, not identical. It is to be noted that in most such cases the "dye droplets" (Golgi vesicles) as well as the Golgi reticulum (ruptured and elongated Golgi vesicles) blacken with osmic acid. Beams and Goldsmith ('30) offered the explanation that a direct chemical reaction might take place between the osmic acid and the vital dye. A good alternative explanation would be one that attributes this affinity to the osmiophilic nature of the Golgi substance of which the vesicles are composed. At the same time, however, it is possible that in some instances an adsorbed cap of vital stain might, as the result of fixation, assume the form of a sphere lying adjacent to a Golgi body and in such a case would indeed be an artificial structure without counterpart in the living, unstained cell. But in my experience, true neoformations have been observed only rarely and with certainty only in tissues that otherwise were obviously abnormal.

Since the chromophilic Golgi vesicles revealed by vital methylene blue staining of the cells of vertebrates closely resemble many of the living Golgi inclusions in molluscs, annelids and arthropods, it is appropriate to conclude that the widely held belief that the Golgi system of the invertebrates typically exists in a much different form than that found in the cells of vertebrates is largely an erroneous conception. It is the result of the failure of many investigators to appreciate the very fluid nature of the Golgi substance in many of its phases and, in addition, the greater tendency of vertebrate Golgi bodies to contact each other when artificially elongated, due perhaps to their initial closer proximity in the living cell. When Golgi bodies occur in life as turgid vesicles they are very prone to collapse or become considerably distorted upon contact with any hypertonic reagent. This is why in-

vestigators have frequently experienced such difficulty in demonstrating the classical Golgi figures in the cells of small marine organisms. In such cases, the fixing fluid is usually more nearly isotonic and the Golgi elements are much more faithfully preserved. It is for this reason that the Golgi bodies are so well represented, for example, in Subramaniam's ('39) study of the endostyle of Branchiostoma. These opinions support to a considerable extent the views held by Bhattacharya ('28), Covell and Scott ('28), Cowdry and Scott ('28), Dawson ('28), Ma ('30), Zweibaum and Elkner ('30), Hall ('31), Ichinomiya ('31), Hirsch ('39) and Hibbard ('42) and, to a lesser extent, that of Parat ('28). Kamnev ('34), using neutral red, reached similar conclusions as the result of methods very similar to those employed in the present study. Many investigators in recent years have realized the inadequacy of the concept of the Golgi apparatus based primarily on the osmic and silver methods.

The above statements do not mean, however, that the apparatus never occurs in life in a form that might be interpreted as reticulum- or net-like. In the active cells of the frog pancreas and in the developing oöcytes and larvae of many invertebrates, the apparatus often occurs as a puddingstone-like mass which, in section, may sometimes resemble a network. However, most such structures have not in the past been referred to as Golgi inclusions, but as "alveolar spheres" (?) (Wilson, 1899), "compound yolk spheres" (Beckwith, '14), "macrosomes" (Chambers, '24), "gray bodies" (?) (Payne, '32), etc. Singh and Boyle ('39) apparently considered them nucleolar extrusions in the oöcytes of the stickleback, something they may very well have been. Such bodies have been referred to by Hirsch ('39) as "polysystems."

In the study reported in this paper, much of the Golgi material of some molluscs, annelids, echinoderms, arthopods, fishes, amphibia, mammals, coelenterates and even protozoa (the results on fishes, coelenterates and protozoa were not described) has been shown to be stainable in life with methylene blue. The conclusion is pertinent, therefore, that the apparatus, given the optimum conditions, will frequently stain with this dye and, unlike the opinion of Hirsch ('39) with regard to neutral red, methylene blue appears to stain the Golgi bodies in most of their active phases. Since, however, certain phases of the apparatus are probably not absorptive, such forms might not prove to be stainable unless the stain were applied in some earlier absorptive phase. This does not, of course, imply that anything that stains with methylene blue in the living cell is Golgi material. Mitochondria in living tectibranch eggs were found to stain pale blue and in many

dying cells the mitochondria have been observed to turn dark blue while the Golgi bodies have shown a tendency to lose the stain to the cytoplasm. Such an effect does not, however, usually result from desiccation. Ludford ('35) experienced considerable success in staining the mitochondria in tissue cultured cells by using heavy concentrations of the stain frequently at a temperature of 37°C. Almost any dead organic matter adsorbs methylene blue. Nucleoli sometimes show a faint trace of stain in what otherwise appear to be perfectly healthy cells. As is well known, living nerve fibers stain with methylene blue as do also the volutin grains in bacteria.

In general, methylene blue appears to color the same structures that are also blackened with osmic acid or with silver in the fixed preparations. However, osmic acid often blackens fats and lipids while these are left colorless by methylene blue. Thus, the methylene blue technique besides staining the Golgi material in the living cell has the additional advantage that it readily differentiates between the Golgi substance and the non-living lipids. The osmic methods are, of course, very useful for material that is to be sectioned, but they would be far more valuable if care were always taken to make the osmic fixatives isotonic with the tissue and ways could be found to avoid, through fixation, distortion of the very fluid Golgi substance.

The methylene blue technique has the disadvantage that in using it one must be continually on the alert for newly-formed dye droplets, but these do not, at present, seem to be as commonplace as was once supposed. As pointed out earlier, however, heavy concentrations of the stain appear to swell the chromophile abnormally, but difficulties of this sort are largely or entirely avoided when weak concentrations are employed. It is possible that some of the chromophilic bodies that appear in the cat pancreas following vital dye treatment are newly formed "excretion droplets," but this certainly cannot apply to the majority of them. Bensley's ('11) term "prozymogen granules" for these bodies is not an altogether inappropriate one, since they are very probably largely responsible for the formation of the zymogen granules here as well as in the frog.

Some of the conclusions reached in this paper may impress the reader as being more than a little reminiscent of the theories of Parat and indeed it appears that, in certain instances at least, Parat has been nearer the truth than many of his critics. Since methylene blue and neutral red frequently stain the same structures there is probably a correspondence between the "vacuome" and the Golgi apparatus in more cases than we are accustomed to believe. The term "vacuome,"

however, is an exceedingly unfortunate one since it implies a series of vacuoles, something the Golgi system certainly is not, at least in the majority of its phases. Neutral red is a much more rapid penetrator than methylene blue, but in my experience the latter has proved to be less toxic for eggs and embryos of invertebrates.

At the same time, it appears that Parat has greatly overestimated the importance of the mitochondria in the formation of specialized products in animal cells. In some instances, Golgi body chromophiles have been called "chondriome" and Golgi body chromophobes have been designated "vacuome," as if these were two distinct cytoplasmic components in temporary close association. My findings support Bowen's view on this question, when he says ('26a, p. 164) it " . . . seems to me practically certain, these two materials are really inseparably related one to the other . . . ". Although Richter ('40), and possibly Norminton ('37), have apparently succeeded in separating these two components with ultracentrifugal force, in the living cell, the chromophobe typically arises within the chromophile during Golgi body development and this close association is maintained until the chromophobe is discharged as a mature fat or protein product.

SUMMARY

1. The classical Golgi figures have been duplicated by vital staining with methylene blue, shrinking the tissue by means of hypertonic salt solution and/or by allowing the material to desiccate on the slide, or by treating the vitally stained tissue momentarily with an osmic fixative, in the malpighian tubules of insects, the salivary glands of the larva of Chironomus, the smooth muscle of amphibians and mammals, the pancreas, liver, kidney, thyroid, anterior lobe of the pituitary and connective tissue cells of the cat, and in the epithelium of the tongue and liver of the frog. It is concluded, therefore, that many of the classical Golgi figures are artifacts caused by fixation shrinkage and/or dehydration of the so-called "dye droplets" or "excretion droplets," which are, in reality, the living Golgi structures. The classical Golgi figures in many tissues can be obtained by the above means within 10 minutes after the removal of the tissue from the body of the living animal.

2. A living Golgi apparatus in the form of a "network" is thought to be a relatively rare condition, but puddingstone-like forms are fairly common.

3. The living Golgi system of most of the vertebrate cells examined has been found to resemble the simple vesicular type of apparatus

found in molluscs. It consists of a series of chromophilic vesicles each of which may enclose a colorless chromophobe. The latter is probably in all cases developing product. The chromophilic pellicle is found to vary in thickness in different species and under different conditions in even the same species, thus causing it to be more opaque in some cases than in others.

4. In the active condition, the acinar cells of the frog pancreas possess a puddingstone-like Golgi mass which is much different from the mammalian Golgi complement of the acinar cells. This is borne out by both osmic and vital staining methods. The discharge of zymogen granules from this apparatus in the frog has been observed, thus confirming on living material Bowen's view of the function of the Golgi apparatus in these cells.

5. Upon collapse, inactive Golgi vesicles appear as rings; active Golgi vesicles elongate and contact each other. The mammalian pancreas and the Chironomus salivary gland are found to be similar in this regard.

6. Golgi bodies of the major phyla of animals and the various classes of vertebrates have been found to be stainable in life with methylene blue. The stain is believed to impregnate the chromophile, i. e., it is absorbed. When used in weak concentration the stain does not appreciably alter the structure of the Golgi bodies, although it is believed that heavy concentrations of the stain may swell the chromophile making it abnormally thickened. Golgi body chromophiles are definitely shown not to be "neoformations" or "excretion droplets."

7. Some evidence is presented to show that the Golgi apparatus has a fluctuating chemical composition, at one time being more lipoidal and at another relatively more protein in character. This fluctuation is believed to be correlated with the nature of the available cellular raw materials and is thought to account, in part, for the variable osmiophily of the apparatus. Not all Golgi bodies are believed to blacken with osmic acid, except perhaps through very prolonged exposure.

8. Golgi droplets in the egg of Arenicola are shown to be relatively solid and composed entirely of chromophilic matter without chromophobe. These are both morphologically and physiologically inactive and similar, relatively solid, homogeneous droplets in other species are believed to be inactive.

9. In general, the views of Hirsch ('39) with regard to the structure and function of the Golgi apparatus are supported.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

(All stated magnifications are approximate)

2 Supravitally stained active forms of Golgi bodies seen in vivo in the Navanax trochophore. Compare with unstained, isolated forms of figure 3 (× 1000).

3 Isolated, unstained Golgi bodies from the Navanax trochophore for comparison with stained forms of figure 2 (× 1000). Golgi body chromophiles appear distinct from chromophobes in the unstained, as well as in the stained, material.

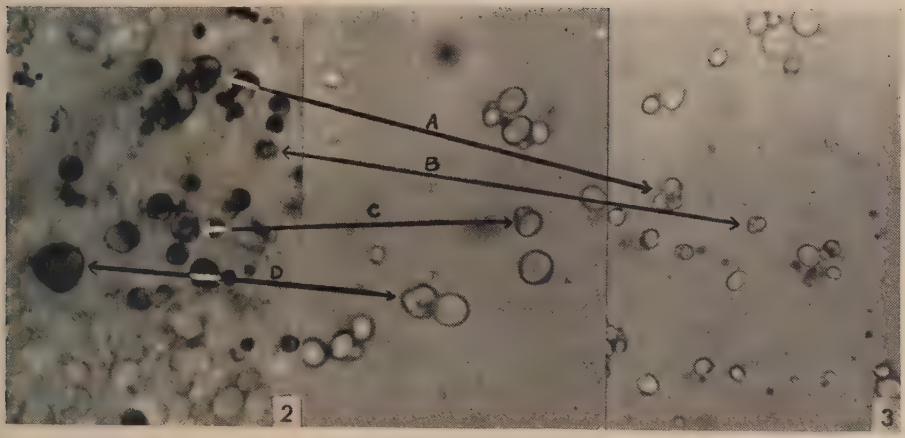
4 Supravitally stained malpighian tubule of Hippiscus, showing localization of stain in the Golgi inclusions (× 100).

5 Same (× 1000).

6 Mann-Kopsch preparation of same for comparison. Arrows indicate Golgi inclusions in which chromophiles can be differentiated from chromophobes (× 1200).

7 Giant yolk-elaborating Golgi body from the embryo of Emerita analoga. Note that the chromophile appears optically homogeneous and that it completely encloses the chromophobe (× 500). In this Golgi body the chromophile has "slipped" somewhat to either side revealing the internal chromophobe.

8 Living gastrula of Strongylocentrotus purpuratus, showing character of Golgi inclusions. Blastopore at lower right (× 600). Vital methylene blue staining.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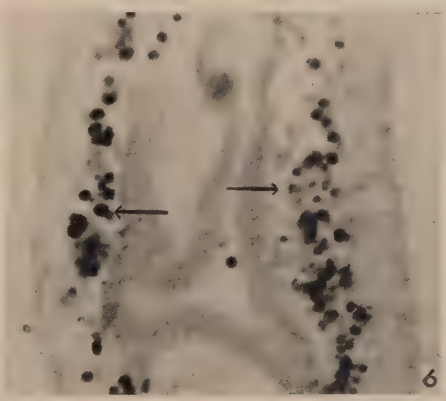
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Blastopore
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PLATE 2

EXPLANATION OF FIGURES

9 Relatively inactive living Golgi bodies of the salivary gland of the larva of Chironomus, 5 minutes after removal from the living larva (× 1000). Supravital methylene blue staining.

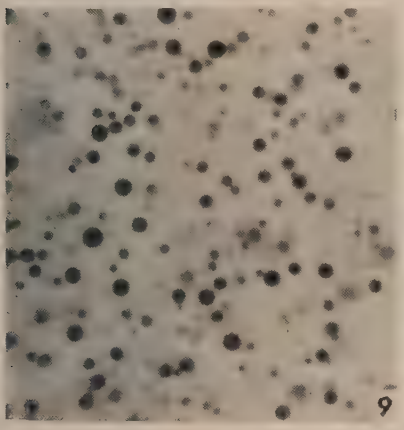
10 Same cell as in figure 9, a few minutes later after treatment with hypertonic salt solution. The Golgi vesicles have collapsed, forming the classical Golgi figures of osmicated preparations (× 1000).

11 Relatively active living cell of Chironomus (× 1000) vitally stained with methylene blue. Note the secretory products (pale) and the relation of some of them to the Golgi vesicles (black).

12 Same cell as in figure 11, a few minutes later after treatment with hypertonic salt solution (× 1000). The Golgi vesicles have ruptured producing the classical Golgi figures.

13 Surface view of the living Arenicola larva, showing methylene blue-stained Golgi bodies as gel-like droplets (× 1000).

14 Part of a section of same for comparison (× 1000). The Golgi bodies undergo little alteration on fixation. Mann-Kopsch technique.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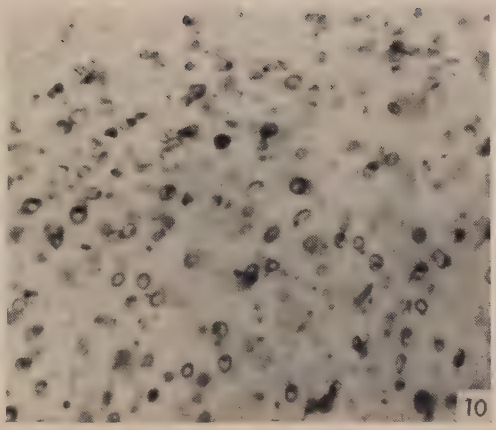
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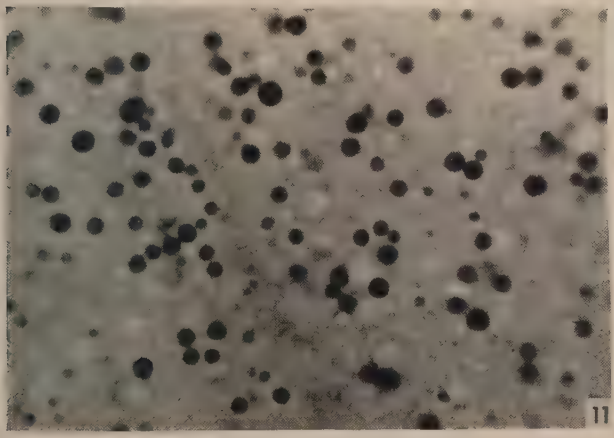
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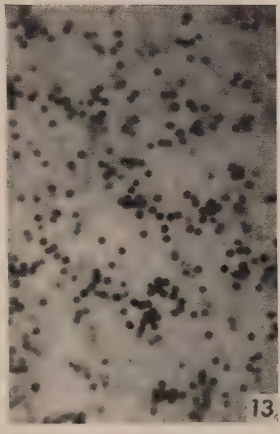
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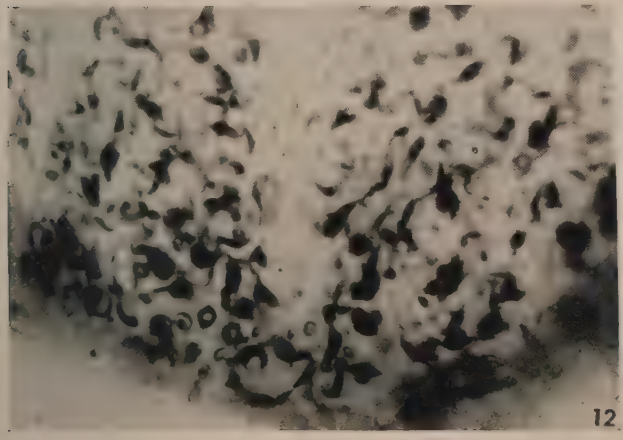
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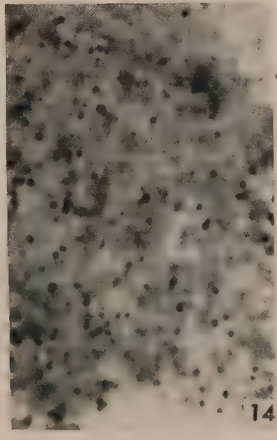
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PLATE 3

EXPLANATION OF FIGURES

15 Living Golgi vesicles of amphibian smooth muscle (× 400). Supravital methylene blue staining.

16 Same, after treatment with hypertonic salt solution (× 400), showing the effect of the reagent on the inclusions.

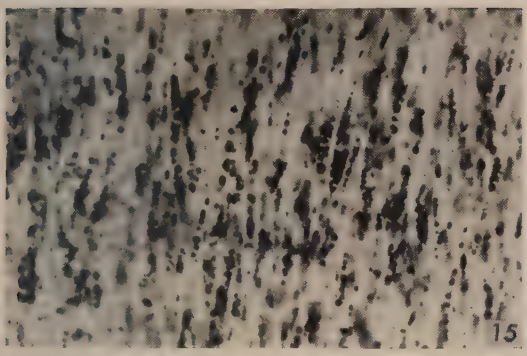
17 Same, but higher magnification (× 1200), to show character of chromophilic inclusions after salt treatment. Note the chromophobe associated with the Golgi body in the center of the composite photograph.

18 Golgi bodies seen in isolated epithelial cells of the frog tongue about 30 minutes after treating the living, vitally-stained cells with hypertonic salt water (× 500).

19 Same (× 1200).

20 Individual cell from material of figure 18 (× 2000).

21 Chromophilic configuration in cells of the convoluted tubule of the cat kidney, about 1 hour after death of the animal (× 2000). Intravitam methylene blue staining followed by treatment with hypertonic salt solution, resulting in an elongation of the Golgi vesicles.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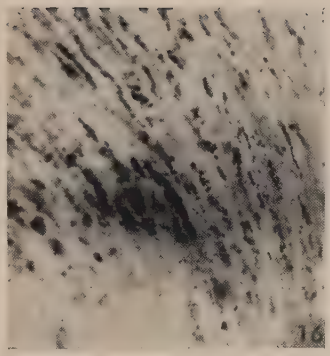
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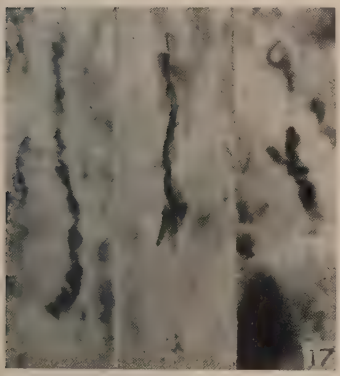
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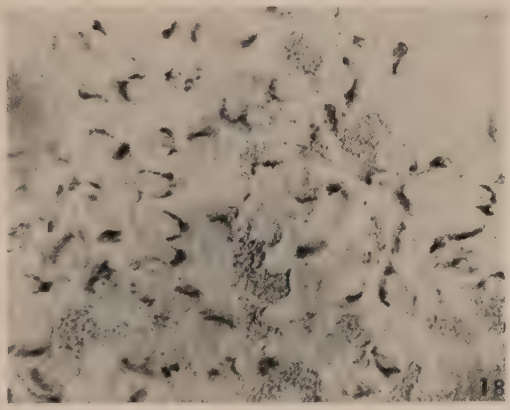
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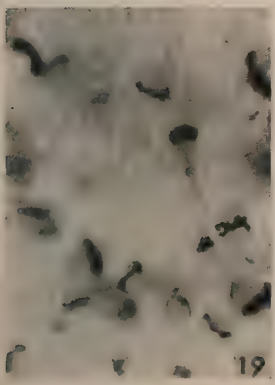
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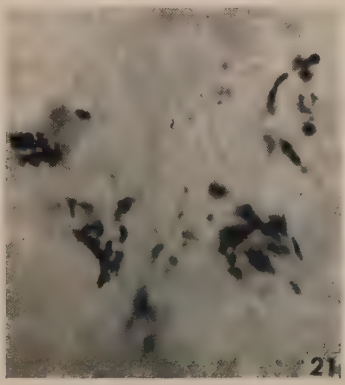
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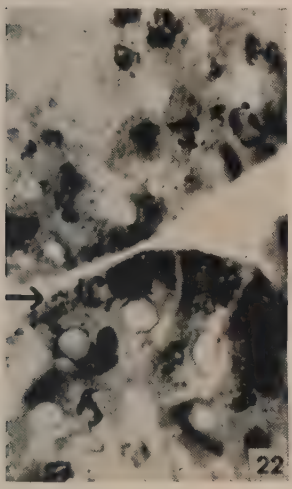
PLATE 4

EXPLANATION OF FIGURES

22 Kolatchev preparation of the alveolar cells of the pancreas of the opossum for comparison with vitally stained inclusions in the guinea pig pancreas of figures 23–27 (× 1000). Arrow indicates tiny osmiophilic rings resulting from the effect of the fixative on the relatively inactive Golgi vesicles.

23–27 Total mounts of alveolar cells from the pancreas of the guinea pig, vitally stained with methylene blue and then briefly treated with Kolatchev fixing fluid (× 1200). The treatment has resulted in classical Golgi figures formed from living Golgi vesicles believed to be relatively active. Photographs taken about an hour after death.

28 Living cells of the epithelium of the frog tongue (× 1200), vitally stained with methylene blue to show the living, undistorted Golgi inclusions.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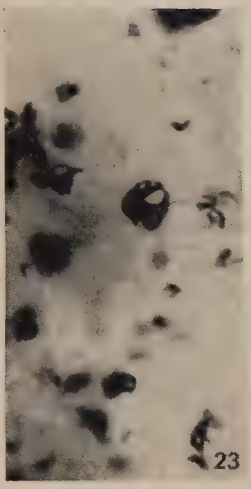
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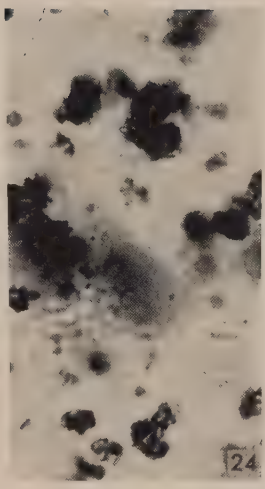
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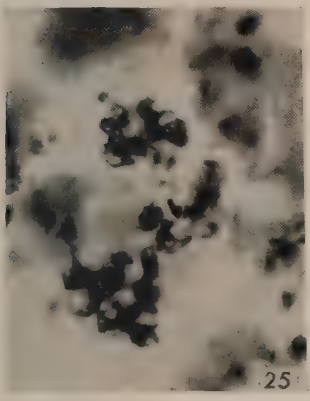
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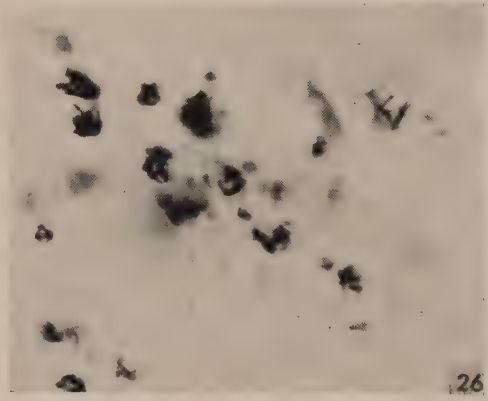
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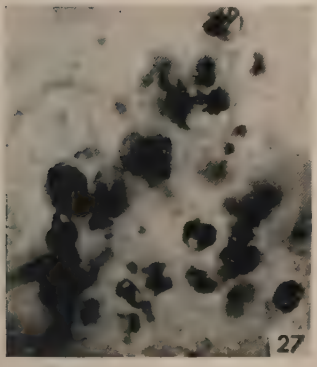
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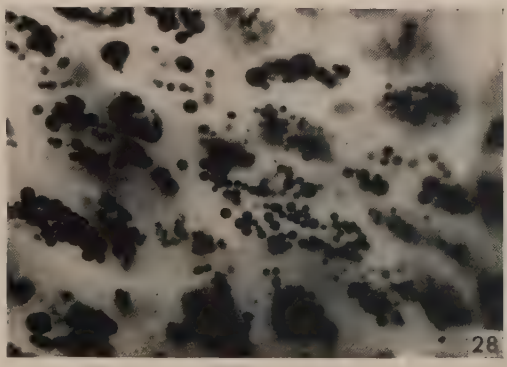
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Contribution No. 235

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*VISION AND GUANINE PRODUCTION IN FISHES**

By Francis B. Sumner

The Scripps Institution of Oceanography

Communicated August 29, 1944

Zoölogists have long known that the purine body guanine, alone or in some combination, is responsible for the silvery appearance of areas of skin in most fishes. It is also a familiar fact that crystals of this substance are contained in special cells, variously called guanophores, leucophores or iridocytes, which respond, as do other chromatophores, to stimuli received through the eyes. The paling of a fish upon a white background results in part from the withdrawal from view of dispersed melanin granules within the melanophores, but also in part from the centrifugal movement of guanine particles within the guanophores. Reverse relations, with respect to both substances, are manifest upon a black background.

That the same stimuli which call forth these rapid adjustments may result, when long continued, in actual large-scale changes in the amount of pigment present has been shown by a number of workers.[1] For melanin, fairly exact relations have now been established in certain fishes between the amount of this pigment and the albedo of the background to which they have been subjected.

For guanine, no exact relations of this sort have hitherto been determined, though several workers have recorded the occurrence of an increase in the number of guanophores or in the quantity of visible guanine in animals kept in white containers.[2] In general, however, guanophores have received relatively little attention from students of chromatophore activity. This is doubtless largely due to their inconspicuousness in comparison with melanophores, likewise to their ordinarily being rendered invisible in histological preparations. The only worker known to me who professes to have undertaken the measurement of guanine in connection with color changes in animals (Meyer, op. cit.) reports a purely negative result, concluding that any possible experimental differences were swamped by the individual variability among the animals studied. This writer fails to state the method which she employed in making these measurements.

After the somewhat extended assays which have been made in this laboratory of the black pigment melanin, in relation to the visual environment, a comparable series of quantitative determinations of the white pigment guanine seemed called for.[3]

A local marine fish, *Girella nigricans*, was employed for this purpose. Two hundred and forty sexually immature[4] specimens were apportioned among 20 painted glass bowls.[5] Of the latter, there were four each of five shades, ranging as follows in respect to albedo, relative to white as a standard.

White	100.00
Pale gray	37.74
Medium gray	17.80
Dark gray	6.90
Black	1.42

Each set of four nearly identical bowls was kept in a separate cabinet, lighted by a 100-watt lamp overhead. Running sea-water was supplied to the bowls.

Of the original number of fishes, 217 were alive at the end of four months, when they were killed with chloroform vapor, dried with paper towels and weighed, commonly in lots of six or seven. These were then dipped into boiling distilled water for three-fourths of a minute and the "skins," including the scales and fins, were removed and dried for several hours in an oven at 100°C. These dried "skins" were also weighed to the nearest tenth gram.

The fishes from the first bowl in each cabinet, which were fewer in number and much larger than the others, were sacrificed for preliminary tests of method. The occupants of the other three bowls of each set were divided into two lots of six or seven fishes each. There were thus six lots from each cabinet (thirty lots in all) which were available for guanine assay. Of these six lots only five have been used.

Thus the fishes under consideration are designated as follows: "1," "2.1," "2.2," "3.1," "3.2," "4.1," "4.2". Of these the "1" lots were used for preliminary tests, while the "4.2" lots have not yet been used at all.

The matter is further complicated by the fact that the solid material of the original "2.1" series was divided into two equal portions ("2.1*a*" and "2.1*b*") and each of these was treated as an independent sample. The results from these are not quite comparable with those from the subsequent series, in that the extraction with acid from the former was less complete than from the latter.

For the methods which I have employed in the extraction and measurement of guanine I am indebted to Dr. George H. Hitchings of the Wellcome Research Laboratories, Tuckahoe, New York, who is reporting upon his

own studies of some of the same material in another article in this issue of the PROCEEDINGS.[6] It is a pleasure to thank Dr. Hitchings for help and advice, kindly given at various stages of this undertaking, though I cannot hold him responsible for every minor step in my procedure. I must also acknowledge the important help of my colleague, Dr. Denis L. Fox, whom I have consulted frequently throughout these studies, as well as using some of the facilities of his laboratory. I must mention, too, the valuable technical assistance of Miss Ruth A. Larson during the earlier stages of these investigations.

The procedure here adopted was as follows:

1. The oven-dried skin was extracted with $N/1$ H_2SO_4, in the proportion of 10 cc. of the dilute acid per gram of the original wet weight of the fishes. Extraction here comprised grinding of the dampened material in a mortar, followed by four cycles[7] of prolonged shaking and centrifuging, in alternation, using a new fraction of the acid for each cycle.
2. Measured portions of this acid extract were heated for one-half hour in 100-cc. centrifuge tubes in a water bath.
3. These were made slightly alkaline with NaOH, using phenolphthalein as an indicator.
4. The contained purines were precipitated with copper sulfate, followed by sodium bisulfite, at boiling temperature.
5. The precipitate was brought down by centrifuge.
6. Precipitate washed twice with boiling water, followed each time by centrifuging.
7. Precipitate redissolved in boiling dilute HCl.
8. Copper precipitated by H_2S, passed through the heated solution.
9. Last precipitate removed from the guanine-HCl solution by filtration through a sintered glass filter.
10. Phenol reagent[8] (Folin-Denis-Ciocalteu) added to the filtrate, followed by Na_2CO_3.
11. Colorimetric determinations made of the resulting blue solution by means of a Bausch and Lomb Visual Spectrophotometer.

The degree of specificity to be expected from this method is discussed by Hitchings and Falco (pp. 294–297 of this issue). These workers have likewise added independent evidence, by the application of an altogether different method, that we are actually dealing here with guanine. This is important, since the procedure which I have employed is not sufficiently specific to distinguish between guanine and certain closely related compounds. It is gratifying to note that the values obtained by Hitchings and Falco, by the use of these two methods agree very closely; and it is further gratifying to note the reasonably close agreement between their figures and mine for the four samples which were assayed independently in the two laboratories

by the copper precipitation method (Black 3.1, 3.2; White 3.1, 3.2). My figures should be compared with those in the middle column of Hitchings and Falco (0.285, etc.).

In making the spectrophotometer determinations, absorption values (logarithmic) were recorded by me at 550, 600 and 650 mμ. Colorimetric

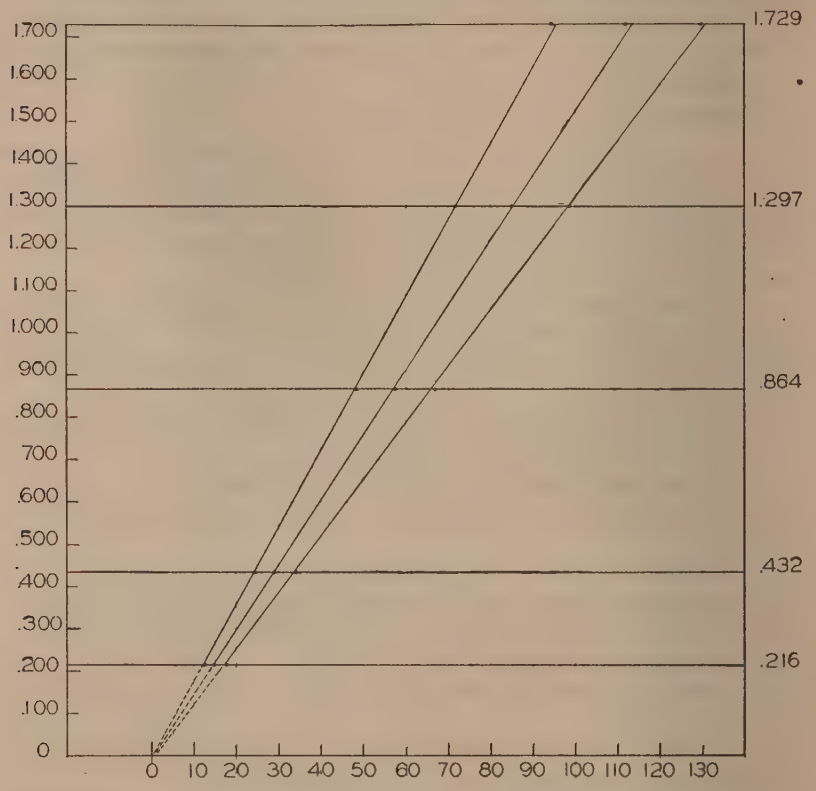

FIGURE 1

Readings with dilutions of standard guanine-HCl solutions. Abscissae = spectrophotometer readings (logarithms of absorption at 550, 600 and 650 mμ). Ordinates = mg. guanine per 100 cc. of solvent.

analysis of this sort obviously requires the previous plotting of graphs, based upon various dilutions of a standard guanine solution (Fig. 1). The values given in the tables, and in figures 2 and 3, are the average values obtained for these three wave-lengths. Five readings at each wave-length were comprised in each determination. It must be added that two to five subsamples of the acid extract were employed in the case of every lot of

fishes, and that the means here presented are based upon all the values thus obtained, commencing with "2.1*a*," except for a few cases in which the treatment had been varied for special purposes.

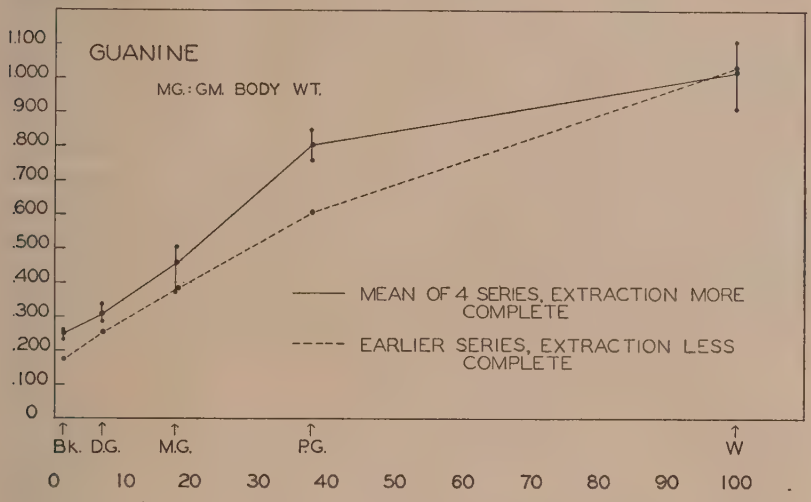

FIGURE 2

Guanine values for *Girella* series. Abscissae = albedos of the five shades of background. Ordinates = mg. guanine per g. of wet weight of fishes. Continuous line based on means of series "2.2," "3.1," "3.2," "4.1." Range of each series indicated by vertical bars. Broken line based upon means of "2.1*a*" and "2.1*b*."

TABLE 1

GUANINE, FIRST EXTRACTION

	BLACK		DARK GRAY		MEDIUM GRAY		PALE GRAY		WHITE	
	MG./G TOTAL WT.	MG./G DRY SKIN	MG./G TOTAL WT.	MG./G DRY SKIN	MG./G TOTAL WT.	MG./G DRY SKIN	MG./G TOTAL WT.	MG./G DRY SKIN	MG./G TOTAL WT.	MG./G DRY SKIN
2.1*a*	0.175		0.243		0.366		0.604		1.036	
2.1*b*	0.179		0.264		0.397		0.618		1.021	
Means	**0.177**		**0.253**		**0.381**		**0.611**		**1.028**	
2.2	0.231	3.61	0.286	4.27	0.371	5.80	0.756	10.65	0.972	13.89
3.1	0.260	3.71	0.293	4.58	0.483	6.90	0.766	11.27	1.084	15.27
3.2	0.239	3.62	0.300	4.48	0.504	6.00	0.851	11.35	0.913	13.63
4.1	0.256	3.77	0.333	5.05	0.467	6.97	0.851	12.33	1.110	16.32
Means	**0.246**	**3.68**	**0.303**	**4.59**	**0.456**	**6.42**	**0.806**	**11.40**	**1.020**	**14.78**

While occasional considerable differences were encountered between subsamples which should have yielded identical results, the mean deviation of such values from their average was 2.5 per cent (extremes: 0 and 6.6 per cent). Such differences are mainly small in comparison with the differences among the various experimental lots which had been subjected to different backgrounds.

The mean guanine values of the various lots of fishes herein discussed are presented in table 1. These values have been computed in two ways. In one column is presented the amount of guanine in the "*skin*" (as defined above) per gram of *total original wet weight* of the fishes. This may not seem to be a very logical value to employ, since the very considerable amount of guanine present in some other tissues of the body was not ordinarily known. In the other column we have the amount of guanine in the skin, in proportion to *the dry weight of the skin.* This last would doubtless be the preferable value to employ, except for the circumstance that the "skin," as here dealt with, was inevitably accompanied by a small but rather variable amount of adhering muscle. This would somewhat affect its weight, probably without correspondingly affecting its absolute guanine content. Thus the values in the first of these columns seem to furnish a fairer index of the changes in the concentration of this substance. Actually, the figures based upon the dry weight of the skin yield a graph very similar to those shown in figure 2.

No calculation of probabilities seems necessary in support of the relations shown by these graphs. Considering the four fully comparable series, "2.2," "3.1," "3.2," and "4.1," we find that the guanine content of fishes from the white bowls is 4.15 times that of those from the black ones, when ratios to total wet weight are considered; 4.02 when ratios to dry skin weight are considered.

In view of the readily visible guanine deposits in the peritoneum of these fishes, it is not surprising that considerable of this substance may be extracted from their "bodies" after removal of the "skins." Only two lots were tested for this purpose, but in these the total amount contained in the "bodies" was absolutely greater than that in the "skins" (1.06 and 1.19:1). Since the latter constituted only about 23 per cent[9] of the dry weight of the fishes, the concentration of guanine was obviously much greater in the skins.

It was early found that even after four "cycles" of extraction, as above described, the fish material continued to yield some substance which gave distinct traces of the blue reaction with phenol reagent and sodium carbonate. The material from the "4.1" series was subjected to these additional extractions, these being carried through four cycles, as had been done originally. The results are shown in table 2.

The mean absolute values of these "second extractions" thus do not differ significantly for the different lots. Indeed, their magnitude appears to bear no relation to the source of the chromogen, a circumstance which is in striking contrast to the figures for the "first extractions," which range from 0.256 to 1.110, as we pass from black to white. This fact suggests that we are dealing with a different chromogen in the earlier and later extractions, and that in these last the amount is not influenced by optical conditions.[10]

I may add that a third extraction (i.e., series of extractions) yielded 0.009 and 0.008, respectively, for "black" and "white" lots (the other lots were not tested). These last figures are of about the same magnitude as was obtained when the H_2SO_4 alone, of the same dilution, was employed, without any known contamination by organic matter. It would seem that all of these guanine measurements are subject to a small constant error, which perhaps could be determined more precisely and applied to the results presented in the tables.

Several earlier writers have stressed the reciprocating relations between guanophores and melanophores, resulting from changes in the background, but without attempting to measure either of the pigments concerned. Thus Kuntz (1917) remarks that "complete adaptation to a white background involves a rearrangement and probably an increase in the number of the guanophores and a material reduction in the number of melanophores in the layer just beneath the epidermis" (op. cit.).

TABLE 2

GUANINE, SECOND EXTRACTIONS

	FIRST EXTRACTIONS	SECOND EXTRACTIONS 1	2	MEAN	% OF FIRST EXTRACTION
Black	0.256	0.021	0.017	0.019	7.4
Dark gray	0.333	0.024	0.020	0.022	6.6
Medium gray	0.467	0.024	0.024	0.024	5.1
Pale gray	0.851	0.022	0.019	0.021	2.5
White	1.110	0.025	0.018	0.021	1.9

Murisier (1920–1921) notes that melanin and guanine seem to be substances which may substitute for one another, the amount of one which is formed being inverse to that of the other. Like melanin, it is formed under the influence of retinal stimuli. Murisier suggests that both guanophores and melanophores form an accessory excretory system, but one which retains its products instead of discharging them.

Millot (1923) sets forth these same ideas at greater length: "The three pigments (melanin, guanine, lipochrome) have almost the same physiological value and belong to the category of waste materials" (trans.). Although guanine is an excretory product, its abundance in the tissues is altogether independent, he asserts, of the food consumed or of the activity or integrity of the renal apparatus.

"Experiment reveals in a number of cases the existence of an equilibrium between the melanin and the guanine such that any diminution of the quantity of one pigment brings about a compensatory augmentation of the other."

While this reciprocal relation between guanine and melanin has been affirmed by several previous writers, it has never, so far as I know, been sup-

ported by quantitative data. Although Millot discusses a method for isolating guanine, he gives no figures to support his claims that the amount of this substance may be influenced by visual stimuli.

In figure 3 of the present paper I have placed in comparison the curve for guanine production in *Girella nigricans* with that for melanin production in this same species, which was obtained under almost identical experimental conditions.[11] The approximate character of the values upon which both of these curves are based has been freely admitted.

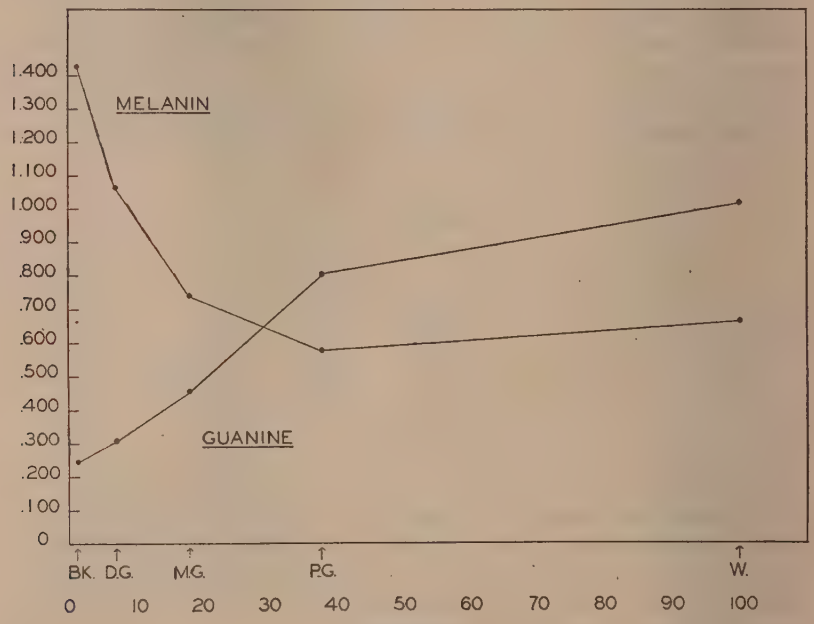

FIGURE 3

Comparison of curves for guanine and melanin in *Girella*. Guanine curve = continuous line of figure 2. Melanin curve is taken from Sumner, 1943.

While the melanin values, from black to pale gray, vary inversely as the logarithm of the albedo, no such arrangement holds for the guanine values. However, the two curves agree in displaying a marked change in their trend beyond pale gray, that for melanin ceasing to fall (indeed, rising slightly) at this point, that for guanine undergoing a marked reduction in the rate of increase. It is, of course, unfortunate that an intermediate albedo between "pale gray" and white was not intercalated in this series, but the relative length of this interval was not realized by eye at the time of mixing the paints.

The outstanding fact revealed by these experiments is that the course of protein metabolism, and the production of two important metabolites, melanin and guanine, are strongly influenced by the visual field of the fish, and influenced in such a way as to make for the animal's concealment.

These fundamental organic processes are thus caused to change their course in the interest of superficial appearance, and this with no apparent effect upon the growth of the fishes. It is true that the strict accuracy of this last statement should be tested more carefully than was done in the present experiments, in which the original mode of selection of the animals, at the time they were distributed to the various bowls, appears to have led to minor size differences which are irrelevant to the question at hand.

That this mechanism for concealment may be of life-saving value has been shown by considerable experimental evidence.[12]

If these two pigments (three, if we follow Millot) actually function as excretory products, it is surely surprising that the necessary excretory balance should be maintained through reciprocal adjustments in the production of substances so widely unrelated chemically. Plainly, there are physiological (shall we not say psychobiological?) problems here which call for extensive future investigation.

* Contributions from the Scripps Institution of Oceanography, New Series, no. 235.

[1] A summary of observations in this field was published by Sumner, *Biol. Rev.*, **15**. 351–375 (1940). Some later results have been reported by Dawes, *Jour. Exper. Biol.*, **18**, 26–49 (1941); by Sumner and Doudoroff, *Biol. Bull.*, **84**, 187–194 (1943); and by Sumner, *Biol. Bull.*, **84**, 195–205 (1943).

[2] Kuntz, *Bull. U. S. Bur. Fisheries*, **35**, 1–29 (1915–1916); Murisier, *Revue Suisse de Zoologie*, **28**, 45–97, 149–195, 243–299 (1920–1921); Millot, *Bull. Biol. de la France et de la Belge*, **57**, 261–363 (1923); Hewer, *Phil. Trans. Roy. Soc. London*, **B215**, 177–200 (1927); Meyer, *Zool. Jahrb. (Abt. f. allgem. Zool.)*, **49**, 231–270 (1931); Sumner and Wells, *Jour. Exper. Zool.*, **64**, 377–403 (1933); Odiorne, *Proc. Nat. Acad. Sci.*, **19**, 750–754 (1933).

[3] I think that guanine may here be legitimately rated as a *pigment*, inasmuch as it actually functions as a pigment, i.e., influences the *color* (in the broad sense) of the animal.

[4] This point is important to note, since it makes certain that no sexual differences in pigmentation can have complicated the picture.

[5] This is the same set of bowls that was employed in the writer's melanin experiments with the same species (*Biol. Bull.*, **84**, 197 (1943)). They were of clear, colorless glass, painted on the outside, and sand-blasted within to eliminate gleams of reflected light.

[6] Hitchings and Falco, *Proc. Nat. Acad. Sci.*, **30**, 294–297 (1944). See also Hitchings, *Jour. Biol. Chem.*, **139**, 843–854 (1941); Hitchings and Fiske, **140**, 491–499 (1941).

[7] Only one cycle in the case of "2.1*a*" and "2.1*b*."

[8] Folin, *Laboratory Manual of Biochemistry*, D. Appleton-Century, 1934; Folin and Ciocalteu, *Jour. Biol. Chem.*, **73**, 629 (1927).

[9] Based upon only one lot of 3 fishes.

[10] Of course, a certain amount of this second chromogen may be supposed to occur in the earlier extractions.

[11] The latter from Sumner, *Biol. Bull.*, **84**, 195–205 (1943).

[12] Sumner, Amer. Nat., **69**, 245–266 (1935); *Proc. Nat. Acad. Sci.*, **20**, 559–564 (1934); **21**, 345–353 (1935).

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Carotenoid Pigments in the Ocean Floor[1]

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INTRODUCTION

Two earlier contributions from this laboratory (Fox, 1937; Fox and Anderson, 1941) dealt briefly with the finding in ocean sediments of what might be called marine *paleobiochromes*, or natural marine pigments of great antiquity from a biochemical viewpoint, although perhaps from relatively recent deposits in the geological sense.

The present discussion is concerned with further investigations of these compounds, their probable origins, history, and chemical significance. These pigments are chiefly fat-soluble yellow, orange, and red members of the carotenoid class and greenish cleavage products of chlorophyll. They occur commonly in the presence of only small amounts of other lipoid-soluble materials, and are regarded as "biochemical fossils" since the time of their burial may, in some strata, antedate the Christian era, the building of Stonehenge, or even the construction of the Egyptian Pyramids.

It is believed that further researches into the occurrence and nature of pigmentary compounds in natural deposits may yield valuable information regarding the biogenic origins of petroleum, coal, shale oil, and the like. In this connection, readers are referred to publications by Treibs (1934) and a review by Lemberg (1938).

Marine carotenoids and chlorophyllous compounds occur together in phytoplankton and other photosynthetic marine plants, and hence in suspended de-

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tritus, feces, marine sapropel, and mud. Carotenoids are also encountered abundantly in many chromogenic bacteria and fungi, and in the tissues (notably integument, liver, and gonads) of many animal species at all depths. Fauna living beneath the lighted zones of the sea are necessarily of carnivorous, detritophagous, or omnivorous habit. Their tissue carotenoids are therefore related metabolically to the polyenes supplied in (1) the bodies of their prey, (2) the incessant rain of algae and organic detritus from the waters above, and possibly (3) the activities of mud-dwelling chromogenic microorganisms as well.

Ingested carotenoids are, in turn (1) partly rejected unchanged from the alimentary tract whence they enter the constitution of bottom mud; (2) partly oxidized metabolically in various degrees; and (3) those stored in tissues may so remain until the animal dies or is eaten.

The continuous supply of organic material from plants and animals of the euphotic zone provides the ultimate food supply for organisms in the depths of the sea. Carotenoids are among the compounds which are less readily assimilated in the alimentary tracts of consumers, and they are less drastically modified by those animals which do assimilate and store them. Thus the manufacture and supply of carotenoids by diatoms, dinoflagellates and other photosynthetic plants, bacteria, fungi, and perhaps also by certain animal species, considerably exceeds the destruction of these pigments. Consequently, some areas of the ocean's floor constitute vast reservoirs for carotenoids and related compounds, which, because of prevailing reducing conditions in the mud proper, the absence of light and the low temperatures, are preserved in a buried condition for centuries of time. In deposits of hundreds or thousands of years' standing, it is doubtless possible that isomerization and other chemical changes may gradually have occurred, with or without microbiological intervention. A number of well known bacterial carotenoids, and a few others of previously unreported absorption spectra have been encountered. Some of the carotenoids met with would appear to have been stored unchanged for ages past. Examples are β- and α-carotene, fucoxanthin, and xanthophylls closely resembling lutein and zeaxanthin. In the light of very recent work by Strain, Manning, and Hardin (1944) it is believed that a considerable part of our sedimentary lutein-like xanthophylls may prove to be diadinoxanthin, extracted from marine diatoms and dinoflagellates, or possibly neodiatoxanthin, an isomer of diatoxanthin from diatoms. Likewise, it is suspected that the zeaxanthin-like xanthophylls encountered in mud are largely diatoxanthin and its isomers found by Strain, *et al.* in diatoms. Sulcatoxanthin, the anemone xanthophyll, is believed by Strain and his associates to be identical with peridinin from peridinians; and violaxanthin and taraxanthin, found respectively in pansy and dandelion flowers, find their marine counterpart in dinoxanthin and its experimental isomer neodinoxanthin, from marine dinoflagellates.

The presence of carotenoids and allied pigments in natural deposits has received attention by a few other workers (1, 17, 20, 2, 31).

This paper deals with the kinds, and in part with relative concentrations of carotenoids in increasingly deep layers of mud, representing suc-

cessively greater ages of deposits. Emphasis is given as well to the possible selective role of marine animals and microorganisms.

Mud samples were studied from regions off the southern California coast, from the Gulf of California, from Mission Bay, and from a freshwater body, Lake Hodges, in San Diego County.

General Methods

Mud-surface sediments were collected with a mechanical snapper; cylindrical columns or cores of deeper lying material were secured with the special coring device of Emery and Dietz (1941).

The marine core-sections examined were invariably highly reducing media, numerous specimens evolving hydrogen or light hydrocarbon gases, accompanied by H_2S, when first collected. All samples were stored in tightly stoppered containers and kept in a cool dark place (often in a refrigerator) pending examination. For analysis, the samples were dried at room temperatures in vacuo, and the fat-soluble colored material was then extracted by repeated treatment with acetone or ethanol. The pigments were subsequently partitioned into (1) epiphasic or carotene-like fractions, selectively migrating to petroleum ether, and (2) hypophasic fractions preferentially soluble in 90% methanol. The hypophasic or xanthophyllic carotenoids were finally driven into fresh petroleum ether by dilution with water. Both fractions were washed free of methanol, dried if necessary, and adjusted to known volumes. Aliquot portions of each were transferred to CS_2 for spectrophotometric estimation. A Bausch and Lomb visual spectrophotometer was employed for reading the absorption of all such pigment solutions. The wave length of 485 mμ was selected, since the aggregated carotenes, as well as the mixed xanthophyll fractions, each showed maximal light absorption at or very close to that point. Epiphasic carotenoids were therefore estimated in "β-carotene equivalents," and hypophasic pigments usually in "zeaxanthin equivalents," in accordance with the apparent preponderating compound in each respective solution. The preponderating xanthophyll was in all probability the diatom pigment diatoxanthin, whose absorption maxima are identical with those of zeaxanthin, but whose specific absorption coefficients have not yet been determined (Strain, *et al.*, 1944).

Tswett chromatographic columns of $Ca(OH)_2$ or MgO were employed for separating individual carotenes from the original epiphasic fraction in petroleum ether; the xanthophylls were resolved into their components by similar adsorption on $CaCO_3$. Chromatograms were developed with benzene if necessary, and each pigment zone was finally eluted with petroleum ether containing small amounts of methanol (Zechmeister and Cholnoky, 1943). The individual pigments were transferred to CS_2 and examined with a Hartridge reversion spectroscope or with the Bausch and Lomb instrument. For diagnostic purposes, it should be emphasized that the Hartridge spectroscope was often found to give slightly higher maxima than were determined with the more accurate spectrophotometer.

Partition of epiphasic pigments between petroleum ether and 95% methanol

demonstrated the absence of all save occasional possible traces of cryptoxanthin. Treatment of epiphasic fractions with hot alcoholic KOH occasionally yielded traces of xanthophyll-like compounds, suggesting the original presence of small amounts of corresponding esters. The amounts were too small, however, to be quantitatively significant in this survey, and may indeed have been products of partial degradation. Likewise, heating hypophasic materials with alcoholic potash only rarely gave rise to faint traces of acidic pigments reminiscent of the astacene group.

Results

Muds from the Gulf of California were far richer both in green chlorophyll derivatives and in carotenoids than were samples of strictly oceanic muds or sediments from nearer shore. Extracts of Gulf muds were usually deep brownish green or brownish yellow due to the presence of chlorophyllous derivatives (intact chlorophyll was never encountered among the mud pigments) and carotenoids, while pigment solutions from oceanic sediments were commonly golden or dirty yellow, yielding much smaller amounts of carotenoids and very little greenish material.

The present studies are confined to the carotenoids. Table I presents a summary of qualitative findings. The relative paucity of pigment allowed only a few classifying properties to be determined. We conducted the treatment as rapidly as possible, and rarely repeated chromatographic purification. Thus it was hoped to minimize alterations or losses by isomerization or oxidation.

Table II lists a number of carotenoids whose absorption maxima resemble those of some of our marine pigments. Thus, while it is not always possible to state that a given pigment encountered in marine mud is, say β-carotene, nevertheless its phasic, chromatographic, and spectroscopic properties may suffice to place it in Group III, which includes β-carotene, or, in the xanthophyll series, zeaxanthin, (or diatoxanthin), etc.

It will be noted in the final column of Table I that, although unusual carotenes resembling such compounds as torulene, flavorhodin, leprotene, or rhodopurpurin, are encountered occasionally, the chief carotenes in sediments belong to (epiphasic) Group III, the β-carotene class, with secondary representation of Group II or α-carotene. Similarly, in the xanthophyll series, while unusual compounds resembling glycymerine or astacene appear in certain samples, we encounter chiefly the hydroxy derivatives of the commoner carotenes, in that members of the zeaxanthin or diathoxanthin class (III) are the chief hypophasic constituents,

with the lutein (diadinoxanthin, neodiatoxanthin) or fucoxanthin group (II) in secondary prominence. In a few instances, xanthophyllic pigments seem to resemble carotenoids of two neighboring groups. In such cases, the assigned group-number represents the pigment's similarity in the position of its second (but chief) maximum; the position of this second maximum (*i.e.*, in the blue-violet) is the more important and more stable criterion.

It is of interest to note the frequent occurrence, in marine sediments, of xanthophyllic carotenoids whose chief absorption maxima in the violet region lie close to 480 mμ, intermediate between the positions of corresponding peaks of lutein or diadinoxanthin (475 mμ) or fucoxanthin (477 mμ) on the one hand, and zeaxanthin or diatoxanthin (482 mμ) or cryptoxanthin and cynthiaxanthin (483 mμ) on the other. This is true to a less extent of some of the carotenes, which thus resemble leprotene, while the corresponding xanthophylls are like antheraxanthin, petaloxanthin or sulcatoxanthin (peridinin). Structural isomerization (*e.g.* diatoxanthin $\longrightarrow$ neodiatoxanthin, according to Strain, *et al.*) is a conceivable factor in the production of some of these marine xanthophylls of Group III during vast periods of time. It is also to be recalled that closely similar xanthophyllic pigments have been encountered in marine invertebrates, *i.e.*, the zooxanthellae-bearing anemones, *Anemonia sulcata* (Heilbron, *et al.*, 1935) and *Cribrina xanthogrammica* (Strain, *et al.*, 1944), another anemone, *Metridium senile*, which is free from algal symbionts (Fox and Pantin, 1941), herbivorous and carnivorous echinoderms (Fox and Scheer, 1941), and occasionally in the liver of another carnivore, the octopus (Fox and Crane, 1942).

Members of this same class of pigments are to be found also in the feces of the California mussel, a consumer of suspended, finely divided marine detritus.

In addition, the results of some preliminary experiments on the bacterial decomposition of one of the commercially important giant kelps, *Macrocystis pyrifera*, are interesting. The fresh blades yielded, as the only major carotenoids, two pigments with absorption spectra closely resembling β- and α-carotenes respectively (although the pigments separated in reverse of the usual chromatographic order) and considerable amounts of fucoxanthin. Finely comminuted tissues of the plant were exposed to mixed bacteria in sea water at initial pH values of 7–8, and were thus incubated in the dark at prevailing room temperatures for periods of from 100 to 186 days. When analyzed, the particulate organic

TABLE I

Spectroscopic Properties of Carotenoid Fractions from Various Sediments

All pigments were examined in CS_2 with a Hartridge reversion spectroscope (H) or a Bausch and Lomb visual spectrophotometer (S). Epiphasic (e), hypophasic (h), and carotenoids passing through the columns (f) are indicated by serial numbers according to relative position on the chromatographic column of $Ca(OH)_2$ or $CaCO_3$ respectively, from top downward (green and excessively dilute zones usually omitted); where colors of zones are recorded, grn. = green; grnsh. = greenish; or. = orange; pk. = pink; r. = red; ylo. = yellow. Where serial numbers are omitted, or followed by a dotted line, the fraction was too scanty for reliable spectroscopic observation.

Location and Sample	Depth *fathoms*	Core section *inches*	Average estimated age *years*	Chromatograph zone of epiphasic (e) or hypophasic (h) fraction	Absorption maxima in CS_2 *mμ*	Remarks	Provisional identification or group (see Table II)
Gulf of Calif.	106	14–18	530	e-3	514.3; 484.1(H)		β-carotene
27°44′N				e-4	513.4; 479.5(H)		leprotene(?)
110°50′W		54–58	1870	e-2	515.6; 482.8(H)		III
(XVI–8)				e-3	516.6; 481.2(H)		III
				h-2	506.2; 481.7(H)		II, III
		102–106	3470	e-2	519.3; 483.8(H)		β-carotene
				e-3	508.6; 479.8(H)		α-carotene
				h-2	506.9; 480.4(H)		II
		134–136	4500	e-3	522.1; 485.4(H)		β-carotene
				e-4	519.5; 483.6(H)		"
				e-5	517.0; 482.1(H)		"
Gulf of Calif.	930	8–12	330	e-3	519.0; 483.1(H)		"
27°17′N				h(whole)	512.4; 481.2(H)	sharp bands	antheraxanthin?
111°08′W				h-3	507.2; 481.6(H)	diffuse bands	II, III
(XVI—10)		24–28	470	e-2	517.2; 482.3(H)	" "	III
				e-3	515.7; 480.1(H)		III
				h-1	512.1; 478.7(H)		II

				h-2	509.9; 478.3(H)	blurred bands	fucoxanthin
				h-3	516.7; 481.9(H)	very sharp bands	sulcatoxanthin or peridinin.
Gulf of Calif. 27°38′N 110°49′W (XVI—27)	209	28–32	1000	e-3	513.4; 478.9(H)	diffuse bands	leprotene
				e-4	514.2; 478.3(H)		"
				h-3	510.6; 480.3(H)		II, III
		64–68	2200	e-2(b)	547.6; 515.8; 481.4(H)	band I thin; band II broad	rhodopurpurin
				e-2(c)	520.3; 485.7; 455.5(H)		III
				e-3	517.0; 480.7(H)		leprotene
				h-1	510.0; 479.0(H)		fucoxanthin
				h-2	509.1; 479.2(H)		"
				h-3	515.7; 481.6(H)	sharp bands; strong absorption coeff.	III
				h-6	510.4; 482.1(H)		III
		96–100	3300	e-2	512.6; 481.0(H)		III
				e-3	568.7; 521.6; 485.5(H)		torulene
				e-4	514.8; 480.0(H)		III
				e-5	516.0; 482.2; 453.7(H)		III
				h-1	513.8; 481.8(H)		III
				h-2	511.8; 481.9(H)		III
				h-3	512.8; 482.5(H)		III
				h-4	514.3; 481.1(H)	sharp bands; strong abs. coeff.	petaloxanthin
		108–112	3700	h-1	512.9; 486.8(H)	very sharp bands	zeaxanthin or diatoxanthin
				h-4	514.8; 480.7(H)	blurred bands	petaloxanthin?

TABLE I—*Continued*

Location and Sample	Depth *fathoms*	Core section *inches*	Average estimated age *years*	Chromatograph zone of epiphasic (e) or hypophasic (h) fraction	Absorption maxima in CS_2 $m\mu$	Remarks	Provisional identification or group (see Table II)
Gulf of Calif.	366	8–12	330	e-2	519.0; 483.4(H)		β-carotene
27°54′N				e-3	518.7; 483.5(H)		β-carotene
111°42′W				e-4	511.3; 477.2(H)	diffuse bands	α-carotene
(XVI—49)		84–88	2870	e-2	506.6; 478.6(H)		α-carotene
				e-3	512.7; 478.0(H)	sharp bands	leprotene
				h(whole)	512.4; 480.0(H)	sharp bands	antheraxanthin?
		154–158	5300	e-2(a)	567.7; 522.3; 486.4(H)		torulene
				e-2(b)	ca 500 in pyridine (H)	acidic on hydrolysis	astacene(?)
				e-3	518.5; 481.2(H)	diffuse bands	III
				e-4	513.7; 480.0(H)	very strong abs. coeff.	III
				h-1	510.9; 480.6(H)	sharp bands	II, III
				h-2	506.5; 476.6(H)		II
				h-3	516.1; 481.2(H)		III
Gulf of Calif.	ca.	—	—	Extr. I:			
28°39′N	800			e-3	522; 487(H); 515; 485; 452(S)		β-carotene
112°59′W							
(XVI—222)				e-6	512; 483(H): 506 481.5(S)	re-chromatographed Spectrum = 506; 478(S)	α-carotene
				e-7	512; 480(H): 508; 480(S)		
				h-1	510; 481(H): 510; 480(S)		II, III

			h-2	500 to 505(H): 507; 490 (S)		.....
			h-4	507(H): 485 to 500 (S)	487 in pyridine (S)	glycymerin?
			Extr. II:			
			e-7	514; 480(H)		III
			h-3	500(H): 501(S)	Strong reactions with $SbCl_3$ or H_2SO_4. Unchanged by hydrolysis treatment. Principal carotenoid in hypophase	glycymerin?
Channel Isl. region off So. Calif. coast 32°37′N 117°21′W (S.L.600)	106	Surface grab sample	e-1 grn.-ylo.	—		
			e-2 lt. ylo.	—		
			e-3 pk.	510; 485(S)		III
			e-4 pk.-or.	520; 485(S)		β-carotene
			e-5 grnsh.	—		
			e-6 r.	—		
			e-7 or.	565; 522; 493(S)		torulene
			e-8 ylo.	513; 482(S)		III
			e-9 or.	—		
			e-10 ylo.	—		
			f-1 ylo.	472(S)		?
			f-2 ylo.	485(S)		?
			f-3 or.	507; 477(S)		α-carotene
			f-4 or.	510; 477(S)		α-carotene
			h-1 ylo.	510; 484(S)		
			h-2 r.	507; 485(S)		
			h-3 ylo.	503; 488(S)		III
			h-4 r.	507; 485(S)		

TABLE I—*Continued*

Location and Sample	Depth *fathoms*	Core section *inches*	Average estimated age *years*	Chromatograph zone of epiphasic (e) or hypophasic (h) fraction	Absorption maxima in CS_2 $m\mu$	Remarks	Provisional identification or group (see Table II)
Channel Isl. region off So. Calif. coast	650	92–102	2400	e-1 pk.	515.4; 484.2(H)		III
				e-2 r.	564.9; 523.7; 492(H)		torulene
32°33′N				e-3 or.	517.2; 480.4(H)		III
117°27′W				e-4 r.	517.0; 483.5(H)		β-carotene
(S.L.496)				e-5 or. (lost)	—		
				e-6 ylo.	503.1; 474.8(H)		flavorhodin
				e-7 r.	515.1; 487.8(H)		III
				e-8 or.	503.2; 473.8(H)		flavorhodin
				e-9 or.	—		
				e-10 ylo.	—		
				e-11 or.	511.7; 484.2(H)		III
				f-1 ylo.	510.6; 469.4(H)		?
				f-2 or.	505.7; 475.4(H)		α-carotene
				f-3 or.	512.6; 478.2(H)		α-carotene
				h-1 ylo.	509.4; 479.4(H)		fucoxanthin
				h-2 ylo.	514.1; 470.9(H)		?
				h-3 or.	516.6; 482.3(H)		III
				h-4 r.-or.	508.4; 469.0(H)		I, II
Mission Bay (fine black intertidal sand)	Low tide level	6–24	—	e-8	513; 481(H)		III
				e-9	513; 479(H)		III
				h-1	502; 476(S)		I, II
				h-2	499; 472(S)	Blue color with 25% HCl; not hypophasic to 70% CH_3OH	I
				h-3	500; 477(S)		I, II
				h-4	485; 470(S)	No color with 25% HCl	?
Lake Hodges (fine black mud)	8	Snap sample from mud surface	—	e-3	471; 428(H)		?
				e-6	516; 484.5(H)		β-carotene
				h(single zone)	508.5; 477.5(H)		lutein or diadinoxanthin

material in the cultures had decreased greatly in weight and had been degraded from small shreds to a fine powdery condition. Cultures which had been rendered anaerobic for the final two-month period

TABLE II

Absorption Maxima (in mμ) of some Carotenoids in CS_2

(From Strain, 1938; Lederer, 1935)

	Carotenes	Xanthophylls
Group I.....	Flavorhodin 503; 472; 441	Violaxanthin 500.5; 469; 440 Taraxanthin 501; 469; 441 Eloxanthin 502; 472 (Dinoxanthin; neodinoxanthin; neodiadinoxanthin)[3]
Group II.....	α-Carotene 507; 476	Pentaxanthin 506; 474; 444 Lutein 508; 475; 445 Fucoxanthin 510; 477 (Diadinoxanthin; neodiatoxanthin)[3]
Group III....	Leprotene 517; 479; 447 β-Carotene 511[1]; 485 Echinenone[2] (520); 488; (450)	Antheraxanthin 512.5; 481; 448 Petaloxanthin 514.5; 481 Sulcatoxanthin (Peridinin) 516; 482; 450 Zeaxanthin 517; 482; 450 Cynthiaxanthin 517; 483; 452 Cryptoxanthin 519; 483; 452 Pectenoxanthin 518; 486; 452 (Diatoxanthin)[3]
Group IV....	Rhodopurpurin 550; 511; 479 Lycopene 548; 507.5; 477	
Group V.....	Torulene 566; 522; 491; 461	

[1] Listed by some writers as occurring as high as 521 mμ with certain instruments (see Smith, 1936).

[2] An epiphasic oxygenated carotenoid from echinoderms (Lederer, 1938).

[3] Parenthesized names are those of newly described xanthophylls (Strain, *et al.*, 1944) whose absorption spectra in ethanol or petroleum ether place them in the indicated groups, but whose CS_2 absorption spectra have not been published.

yielded especially interesting results. No significant pH changes in these were manifest, although considerable amounts of H_2S were apparent, and motile bacteria were observed. β-Carotene remained in

apparently unchanged quantities, but fucoxanthin seemed to have been chemically changed in part to xanthophylls of Group III, spectroscopically similar to antheraxanthin and petaloxanthin, with absorption maxima (in CS_2) of 510, 480 mμ and 513, 481 mμ. In one 100-day aerobic culture, a xanthophyll resembling taraxanthin (or dinoxanthin, neodinoxanthin or neodiadinoxanthin discussed by Strain, *et al.*) was detected, with absorption maxima at 499, 470, 443 mμ. This was accompanied by a unique, unstable xanthophyll (477, 448 mμ).

In the anaerobic cultures, pigments resembling the antheraxanthin class appeared perhaps in partial replacement of fucoxanthin. Thus the preliminary anaerobic experiments, designed to simulate somewhat conditions in the ocean floor, resulted, through a microbiological agency, in a carotenoid picture similar to some which have been encountered in marine sediments.

In the foregoing connection, some observations of Clarke and Mazur (1941) on the microbial reduction of diatom lipids are of interest. Analyses of various diatom catches and cultures revealed a high preponderance of uncombined fatty acids over all other constituents of the lipid fractions. Furthermore, decomposition of diatom sludges by marine microorganisms over periods of 6 months yielded a great fall in the proportion of free acids, often with a marked increase in the hydrocarbon fraction. The data reveal higher proportions of hydrocarbons, in marine mud and in diatom sludges decomposed by bacteria under air or under nitrogen, than in fresh diatoms. Thayer (1935) observed that carotenoids and chlorophyll derivatives were among the most resistant protoplasmic components in the anaerobic decomposition of diatoms. He suggested that pigments may contribute, at least in a minor way, to the genesis of petroleum. ZoBell (1944) has considered the potential role of lipolytic bacteria in the genesis of hydrocarbons allied to those encountered in petroleum.

Table III lists values for epiphasic and hypophasic pigments in various sediments of different ages. The primary arresting fact is the high proportion of carotenes over xanthophylls in all sediments, as compared with representative marine plant and detrital materials (Table IV). At Stations S.L. 600 and S.L. 601, the total carotenoid content of marine sediment underwent a decrease with depth down to 4 feet, representing an aggregated accumulation of more than 7 centuries.[4]

[4] Geological information from Professor Francis P. Shepard, personal communication.

TABLE III

Quantities of Carotenes and Xanthophylls in Marine Sediments of Different Ages

Carotenes are expressed in terms of β-carotene equivalents, xanthophylls in zeaxanthin units unless otherwise specified

Location and Sample	Depth	Core section	Av. estimated age	Carotenes *mg./100 g. (dry wt.)*	Xanthophylls *mg./100 g. (dry wt.)*	Total carotenoids *mg./100 g. (dry wt.)*	Carotenes *per cent*
	fathoms	*inches*	*years*				
Channel Isl. region off So. Calif. coast 32°37′N 117°21′W (S.L. 600)	106	Surface grab sample	—	0.13	0.04	0.17	76.5
Same as S.L. 600 (S.L. 601)	106	1–12	15–180	0.20	0.04	0.24	83.2
		12–24	180–360	0.05	0.01	0.06	83.2
		24–36	360–540	0.04	0.01	0.05	80.0
		36–48	540–720	0.02	0.01	0.03	66.6
Off Pt. Loma, Calif., 32°37.6′N 118°09′W (Fox 1937)	1096	1–4	—	0.07	0.13	0.20	35.0
Channel Isl. region off So. Calif. coast 32°33′N 117°27′W (S.L. 496)	650	96–102	2500	0.24	0.05	0.29	83.0
Gulf of California 27° 41′N 110° 55′W (Fox and Anderson, 1941)*	364	180–195	6000	0.42	0.19 (lutein units)	0.61	69.0
Mission Bay (fine black intertidal sand)	Low-tide level	—	—	—	—	0.01	(mostly carotenes)
Lake Hodges (fine black mud)	8	Surface grab sample	—	0.11	0.18	0.29	37.8

* The figures in this table are corrected; those published in the earlier paper cited (p. 335) are 10-fold too high due to a misplaced decimal.

The proportion of carotenes in total carotenoids rose from 76.5% at the mud surface to 83% in the first 2 feet of the core; the succeeding 3 and 4-foot depths of sediment showed a steady decrease in the preponderance of hydrocarbon types over oxidized carotenoids, although even at the 4-foot depth, wherein the total carotenoid content was about half that at the surface, carotenes still constituted 66%. Local differences are

TABLE IV

Quantities and Relative Proportions of Carotenes and Xanthophylls in Marine Plants and Other Marine Materials

Values are expressed in mg. per 100 g. dry material unless otherwise specified.

Material	Carotenes	Xanthophylls	Total	Carotene *per cent*	Reference
Prorocentrum micans	0.26	2.26	2.52	10.3	Scheer, 1940
Nitzschia closterium	65.9	578.5	644.4	10.3	Pace, 1941
" "	—	—	270, 296	....	Fox (unpubl.)
Sea-water detritus (from sea-water settling reservoir via lead pipe to laboratory)	—	.....	0.123 mg. (from 4000 liters)	18.3	Fox, 1937
Mussel feces	3.3	51.0	54.3	6.1	Fox, *et al.*, 1936
" "	2.0	.....	12.5	16.0	Fox (unpubl.)
Feces of mussels on *Prorocentrum* diet	0.75	4.38	5.13	14.6	Scheer, 1940
Fucus sp.	8.9	25.6	34.5	25.7	Willstätter and Page (from Zechmeister, 1934)
Dictyota sp.	5.7	31.3	37.0	15.4	"
Laminaria sp.	0.6	11.9	12.5	4.8	"
Rhizosolenia styliformis + *Biddulphia sinensis*	35.0	65.0	100.0	35.0	Gillam, El Ridi, Wimpenny, 1939

exemplified by data collected from a core taken at Station S.L. 496 at a depth of 650 fathoms. Here at a depth of some 8 feet (estimated age of deposit about 2500 years), the total carotenoid content of vaccum-dried sediment even exceeded that of the first foot of the other core, *i.e.*, 0.29 mg.%, the proportion of carotenes being 83%. Black, reducing sediment (containing H_2S as in the other samples) collected from the low-

TABLE V

Approximate Quantities of Xanthophylls and Carotenes in Various Marine Species

Expressed in mg. per 100 g. wet tissue

Species	Xantho-phylls	Caro-tenes	Approx. carotenes %	Reference
Fishes				
Fundulus parvipinnis....	0.77 to 1.2	0	0	Sumner and Fox (1933, 1935a)
Girella nigricans.....	ca 1.4	0	0	Sumner and Fox (1935b)
Gillichthys mirabilis.....	0.41 to 0.62	0	0	Sumner and Fox (1933)
Cymatogaster aggregatus.	ca 0.27	0	0	Young and Fox (1936)
Hypsypops rubicunda....	very concentrated in skin	0	0	Fox (1936)
Mollusks				
Mytilus californianus				
male body.....	2.18	0	0	Scheer (1940)
male gonad.....	6.05	0	0	"
female body.....	4.83	0	0	"
female gonad.....	11.34	0	0	"
Paroctopus bimaculatus				
ink.....	0.55 to 0.7	0	0	Fox and Crane (1942)
*Echinoderms**				
Dendraster excentricus..	0.049	0.157	76	Fox and Scheer (1941)
Strongylocentrotus franciscanus.....	0.025	0.170	87	" " " "
Strongylocentrotus purpuratus				
female intestine.....	6.04	2.99	33	" " " "
female gonad.....	0	2.00	100	
male intestine.....	5.65	2.41	30	
male gonad.....	0	0.69	100	
Lytechinus pictus				
whole females.....	0.35	0.49	58	" " " "
whole males.....	0.43	0.87	67	
female intestine.....	4.18	4.48	52	
female gonad.....	0.28	0.86	75	
female test.....	0.01	0.01	50	
male intestine.....	1.52	3.38	69	
male gonad.....	0.53	4.48	90	
male test.....	0.01	0.01	50	
Stichopus californicus..	0.015	0.014	48	" " " "
Astropecten californicus.	0.72	0.044	5.8	" " " "

TABLE V—*Continued*

Species	Xantho-phylls	Caro-tenes	Approx. carotenes	Reference
Patiria miniata	0.86	0.05	5.5	Fox and Scheer (1941)
Pisaster ochraceous				
skeleton + skin	0.24	0.23	49	" " " "
pyloric caeca	11.45	0.56	4.7	
Pisaster giganteus				
skeleton + skin	0.90	0.07	7.2	" " " "
pyloric caeca	1.37	0.40	22	
Ophiopteris papillosa	>3.39	0	0	" " " "
Ophiothrix spiculata	>1.60	0	0	" " " "
Ophiothrix rudis	>1.40	0	0	" " " "
Annelid				
Thoracophelia mucronata	0	0.38	100	Sumner and Fox (1933)
Anemone				
Metridium senile				
red variant	14.96	0**	0**	Fox and Pantin (1940)
white variant	1.76	0	0	" " " "
orange variants, brown variants	(highly variable, but carotenes in minority when present)			" " " "

* It should be emphasized here that, among the echinoderms, notably such members of the echinoid class as the urchins *Strongylocentrotus* and *Lytechinus*, the ketonic carotenoid *echinenone* was demonstrated by Fox and Scheer (1941) to constitute a considerable portion of the epiphasic pigments, placed therefore in the hydrocarbon group with the carotenes. Echinenone seemed to be present also in the holothurian, *Stichopus*, and in one asteroid, *Astropecten*. The presence of echinenone in the carotene fraction would of course diminish the relative proportion of true carotenes listed in certain echinoid tissues in the above table.

** Occasional red individuals were found to contain a little carotene.

tide shoreline in Mission Bay yielded carotenoids in amounts of only 0.01 mg.%. The lake sample yielded but four epiphasic carotenoids against 14 such fractions in the marine material; β-carotene was present in both kinds of deposit. The hypophase from the lake mud yielded only a lutein-like xanthophyll, while in the marine core (S.L. 601) the four chromatographic zones from the hypophase were dissimilar from this pigment (Table I).

A survey of the carotenoids present in some representative marine plants and animals reveals a striking reversal in the ratio of carotenes

to xanthophylls encountered in marine sediments. For in the green plants of the sea, the oxygenated class of carotenoids preponderates greatly over the hydrocarbon class (Table IV), and the same seems to be true of the great majority of marine animals. Indeed, xanthophylls are the exclusive carotenoids of some fauna, while we have encountered to date only one marine animal species, *i.e.*, the worm *Thoracophelia*, which stores only carotenes (Table V).

Table V shows a list of several marine animal species, most of which were found to contain xanthophylls only or in preponderance. Attention is called also to the important investigations of Lederer (1938), who examined the carotenoids of an extended list of organisms, and found, in tissues of numerous species of fish, tunicates, mollusks, and crustaceans, that oxygenated carotenoids were greatly preponderant, and in some instances the only polyenes present. In the gonads of the sea-urchin *Strongylocentrotus lividus*, Lederer encountered appreciable amounts of α- and β-carotene, accompanied by echinenone and a number of xanthophylls, predominantly the newly described xanthophyll pentaxanthin. In the sponges *Suberites domuncula* and *Ficulina ficus* he suspected the chief carotenoids to be members of the hydrocarbon group, *i.e.*, torulene, α-, β- and γ-carotene.

Karrer and Solmssen (*cf.* Lederer, 1938) obtained astacene from the sponge *Axinella crista-galli*, while Drumm and O'Connor (1940) found, in another sponge, *Hymeniacidon sanguineum*, no astacene, but echinenone and γ-carotene.

Discussion

A. Approximate quantitative relationships

We may now draw some provisional conclusions regarding the fate of carotenoids in the sea. Resulting inferences may apply to channels of diagenesis involving hydrocarbons and other relatively refractory organic materials of marine sediments.

Krogh (1934) provides the average figure of 5.4 g. of total organic matter per cubic meter of sea water in the ocean as a whole. Trask (1939) records the approximate value of 2.5% of organic matter in nearshore sediments. He considers the average rate of sedimentation to be about 1 cm. in 25 years, and the density of the deposited material to be about 2.0. He points out that this would represent some 20 g. of organic matter accumulating on each square meter of bottom per year, or 2% of the estimated 1000 g. of organic compounds assumed to have been

synthesized over the annual period in the average 2000 fathoms of water above each square meter of ocean floor.

The concentration of pigments in ocean waters has not been estimated in an accurate way, but an approximation may serve to indicate the order of magnitude. Gillam, El Ridi, and Wimpenny (1939) provide data which indicate a general average of 0.0076 mg. of carotenoids per cubic meter of North Sea water. However, their use of a Hensen silk net of 60 meshes per inch would allow much of the very fine material to escape analysis. The additional stated fact that astacene was not measured would add to the departure from true values.

The occurrence of carotenoids and chlorophylls in certain ratios among marine algae (Pace, 1941; Zechmeister, 1934), and the estimations of Graham (1943) and others of green pigments in ocean samples might suggest the use of such indices as rough approximations of parallel carotenoid content. However, chlorophyll is readily decomposed by oxidation, hydrolysis, or other processes, whether in ordinary chemical ways or at the agency of bacteria or herbivorous animals. Therefore, serious inaccuracies might be involved in any attempts to estimate carotenoids in terms of unidentified and highly variable green pigments grouped together as "chlorophyll."

No extensive carotenoid determinations have been carried out on paper-filtered marine detritus, but some analyses have been made of fecal material from the California mussel, which derives its nutrition by filtering out and swallowing finely particulate and colloidally suspended marine detritus (*cf.* Scheer, 1940; Fox and Coe, 1943). Its feces often yield small, sometimes negligible quantities of chlorophyll derivatives, but never fail to contain varying but appreciable amounts of carotenoids. These have been estimated in a number of fecal samples. From Table IV, for example, a rough average value might be taken as about 24 mg.%, equivalent to about 80 mg. per 100 g. of fecal *organic* matter (Fox and Coe, 1943), of which carotenes were present in about the proportion often encountered in phytoplankton, *i.e.*, 15% of total carotenoids. Analysis of mussel fecal material has shown that about 12% of the organic matter may be lipid; thus the concentration of carotenoids in *lipids* of marine detritus would approach 0.66%. Bearing in mind that the quantities of carotenoids in marine plankton and detritus must be highly variable with season, locality, and other environmental factors, and that some of the xanthophyllic pigments ingested are assimilated by the mussel (Scheer, 1940), we may nevertheless

utilize carotenoid values in mussel feces as representative of the order of magnitude in materials that constitute the source of organic matter in bottom sediments.

The approximate data should reveal the general order of magnitude applying to the standing crop, the new crop, and the fossil crop of organic matter and carotenoids in the sea, as follows.

Standing crop: If the average quantity of organic matter is 5.4 g. per cubic meter (Krogh), and the average quantity of carotenoids 80 mg. per 100 g. organic matter, then a column of ocean water one square meter in cross section and 2000 fathoms (*i.e.*, 3650 meters) high should contain some 19,710 g. of organic matter, including about 2365 g. of lipids, and 16 g. of carotenoids (14 g. of xanthophylls and 2 g. of carotenes).

Annual crop: If, according to Trask, 1000 g. of new organic matter are synthesized per year in a column of ocean water of the above dimensions, this value would correspond to approximately 120 g. of lipids, including 0.8 g. of carotenoids (*i.e.*, 0.68 g. of xanthophylls and 0.12 g. of carotenes).

Fossil crop: Since no analytical studies could be made upon the composition of the uppermost few millimeters of mud, no information is available regarding the *annual* fossil crop. Some comparisons may be drawn, however, between the standing crop of suspended and that of long buried materials. A value of 0.25 mg. carotenoids per 100 g. dry sediment represents, on the basis of Trask's estimated 2.5% of organic matter in such muds, about 10 mg. carotenoids per 100 g. organic matter. We found about 0.05% of lipid material in samples of marine sediments, or approximately 2% of the organic residue. This would mean 500 mg. carotenoids (*i.e.*, 400 mg. carotenes and 100 mg. xanthophylls) per 100 g. sedimentary lipids.

In resumé, these rough approximations lead to some interesting provisional conclusions. If the organic fraction of marine detritus, represented by mussel-feces, contains 12% lipids, 68 mg.% xanthophylls and 12 mg.% carotenes, and if the organic portion of bottom sediments commonly yield 2% lipids, 2 mg.% xanthophylls, and 8 mg.% carotenes, this process of early fossilization would represent a loss of 83% in lipids, about 97% of xanthophylls, and only 33.3% of carotenes. But, whereas the lipid fraction would appear to have undergone a decrease of 83% in its xanthophyll content, *i.e.* from 566 mg.% down to 100 mg.%, the carotenes seem to have remained more refractory than either the xantho-

phylls or the lipids themselves, for their concentration of as much as 400 mg.% in sedimentary lipids is greater by some 4-fold than their estimated concentration in the lipids of suspended detritus. It is perhaps too early to extend into a generalization this apparent sparing of hydrocarbon carotenoids at the expense of the other lipids.

B. Implications Relative to Petroleum Formation

Marine lipid materials are commonly regarded as potential fore-runners of petroleum compounds. With this in mind, we may make a brief comparison between lipoid-soluble components in the marine sediments which we have examined and the oil content of earth in representative oil wells.

One cubic meter of marine mud weighing 2000 kg. or 4400 lbs. contains approximately 0.05%, or 1 lb. of lipoid-soluble constituents per ton. A good oil well is expected to bear at least 20 gal. or about 125 lbs. of oil per ton of solids. Representative marine sediments would therefore appear to bear roughly 1% of lipids potentially contributory to the genesis of natural oil. In spite of such a low figure, it is conceivable that some sediments may give up any excess oily material which then finds its way through fissures into pockets or masses. As an example, one marine sediment core examined in this laboratory yielded both chlorophyll degradation products and carotenoids at the 6 inch, 74 inch, and 80 inch levels (representing strata of 600, 7400, and 8000 years old respectively). The lipid contents of these three core-sections were 0.06%, 0.05%, and 0.04% by dry weight respectively. But an intermediate section of the same core, 44 inches beneath the mud surface (estimated age 4000 years), yielded the ten-fold quantity of lipid material. This sample contained greenish chlorophyllous material of the same spectral properties as that encountered in the other core sections, but, unlike them, bore no trace of carotenoids. Instead it yielded a pigment of blue-green fluorescence, identical in chromatographic behavior and spectral absorption with a pigment obtained from a sample of crude California petroleum (Fox and Anderson, 1941). The absorption spectrum of this pigment (in $CHCl_3$) agreed closely with that of a California asphalt pigment (in pyridine-ether) isolated by Treibs (1934).

Continued attempts to correlate findings of plant porphyrins, total lipoid-soluble materials and carotenoids in marine sediments with similar studies on oil-bearing deposits are of manifest importance.

C. Qualitative Features

The properties of numerous carotenoids in marine sediments suggest their derivation directly from marine photosynthetic plants. Such pigments are the commoner carotenes, fucoxanthin, and certain newly described phytoplankton xanthophylls spectroscopically similar to zeaxanthin and lutein. Sulcatoxanthin, or peridinin, is common in marine mud. Animal carotenoids resembling glycymerin and astacene are occasionally encountered, while another group of pigments found characteristically in saprophytic plants suggest sedimentary contribu-

tion by such organisms. Torulene (from red yeasts) and other carotenes such as rhodopurpurin and flavorhodin (both from Rhodovibrio bacteria) are examples. It is noteworthy that these *hydrocarbon* pigments, ordinarily encountered in Rhodovibrio were not accompanied by xanthophyllic derivatives such as rhodoviolascin or rhodopin found in the same group of organisms.

Several chemical and biochemical agencies may combine in effecting the more rapid destruction of xanthophylls than of carotenes in the ocean's floor. In the first place, xanthophylls are more sensitive than are carotenes to atmospheric oxygen. In solutions or in solid form they more readily undergo autoxidation with bleaching (Willstätter and Mieg, *cf.* Zechmeister, 1934). The prominent marine xanthophyll, fucoxanthin and its isomers are especially susceptible to alteration by heat or alkalies (Strain, *et al.*, 1944) and may be bleached or otherwise altered by long standing. Secondly, it is known that the majority of marine animals investigated to date store chiefly xanthophylls rather than carotenes in their tissues. Exceptions may be encountered among bottom-dwelling worms, sponges, and echinoids. The xanthophyll-selecting animals doubtless oxidize some of the assimilated carotenoid instead of storing all of it. Filtering or bottom-feeding detritophagous animals which selectively assimilate xanthophylls, rejecting carotenes in their feces, could thus manifestly implement a gradual proponderance of the hydrocarbon type over the oxygenated type of carotenoid in marine sediments.

Finally, as has been suggested, microorganisms living in the anaerobic marine muds may be able to reduce certain xanthophylls to a lower oxygen content, or even to carotenes.

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Summary

1. Sediment samples of the ocean floor contain substantial amounts of carotenoids and chlorophyllous degradation products laid down many centuries ago.

2. In contrast to green marine flora, likewise nearly all marine fauna

investigated, as well as suspended detritus of the sea, all of which yield xanthophyllic compounds in great excess over the carotene type, the relative proportions of these two carotenoid classes are strikingly reversed in marine muds.

3. Among the polyene hydrocarbons, β-carotene consistently seemed to preponderate, with α-carotene in secondary prominence, and various unusual carotenes, associated with bacteria, fungi, and certain sponges and ascidians, in lesser quantities and of more sporadic appearance.

4. Of the xanthophylls, zeaxanthin or more probably diatoxanthin, appeared to be the chief representative, followed by other alcoholic polyenes similar to antheraxanthin, petaloxanthin, sulcathoxanthin, lutein, fucoxanthin, and other xanthophylls described as characteristic of marine phytoplankton.

5. Of possible agencies operative in the selective storage of the hydrocarbon types over the oxygenated class of carotenoids in marine materials, several tentative suggestions are offered: (1) greater rates of autoxidation of xanthophylls than of carotenes in regions containing dissolved oxygen; (2) selective assimilation of xanthophylls and fecal rejection of carotenes by the majority of marine animals; (3) possible chemical reduction of xanthophylls to polyene hydrocarbons by certain marine bacteria in anaerobic environments.

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PIGMENTS IN THE COELENTERATA

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Considering the brightness and variety of the colours of marine invertebrates it is surprising that we still know so little of their biochemical origin and of their biological importance to the animals that possess them. Of no group is this so evident as of the Coelenterata, and the occurrence of large amounts of different pigments in their thin tissues raises the problems of the origin and significance of colour in the most forcible manner. The object of this article is to gather together the limited information we have about these pigments and to draw attention to some of the interesting problems which they present.

Some of the references to the scattered literature are found in the books of Newbigin (1898) and Verne (1926, 1930), and in a recent review by Lederer (1940). A paper by Payne (1931) contains some rather serious inaccuracies with reference to some of the early work. Confusion has sometimes arisen in the literature as to the identity and systematic relations of the animals studied; and as our studies (Fox & Pantin, 1941) of the pigments of the plumose anemone, *Metridium senile*, impressed on us the importance of correct identification not merely of the species but also of the variety investigated, we have wherever possible named species in accordance with Stephenson's monograph on British sea anemones (1928, 1935) and Mayer's *Medusae of the World* (1910). Alternative names used by authors in the works referred to are placed in brackets.

I. MISCELLANEOUS PIGMENTS

Some of the commonest colours in coelenterates are due to pigments whose nature is still uncertain. Among these are the deep blue and the red-brown pigments responsible for the vivid colour of many pelagic coelenterates, such as the Scyphozoa and the Siphonophora, particularly in such predatory forms as *Cyanea* and *Physalia*. In this class we know most about the pigment which is present as minute granules in the scyphozoan *Rhizostoma cuvieri* (Krukenberg, 1882*b*; Blanchard, 1882; Colasanti, 1888). Krukenberg's work was confirmed and greatly extended by von Zeynek (1913). The pigment has three absorption bands, in the red, yellow and green. It is soluble in water, but it is not very stable. It turns violet with alkalis and red in acids, but the latter is an irreversible change. The pigment is altered to a red substance at 50° C. and it is destroyed altogether on boiling. A number of organic solvents such as alcohol, acetone, chloroform, etc., throw it out of solution as a red precipitate. Von Zeynek purified it by fractional precipitation with ammonium sulphate and obtained the following evidence of its composition: C, 51·69 %; H, 7·14 %; N, 10·38 %; S, 1·26 %; ash, 4·0 %. The reactions of the pigment indicate that it is a protein. Krukenberg called it 'cyanein', while Colasanti and von Zeynek refer to it as 'zoocyanin'.

Pigments of very similar properties occur in other Scyphozoa, such as *Aurelia* sp., *Cyanea* sp. and *Cassiopeia borbonica* (M'Kendrick, 1881; Colasanti, 1888; MacMunn, 1890). Colasanti did not, as the title of his papers leads one to suppose, investigate any hydromedusae. He did, however, find a pigment apparently identical with that of *Rhizostoma* in the siphonophores *Velella spirans*, *Porpita mediterranea* and *Physalia pelagica*. Some further observations on the pigment of *Velella* have been made by Haurowitz & Waelsch (1926) and by Kropp (1931). The spectra of all these pigments seem to vary in the distinctness of the absorption bands. Haurowitz & Waelsch noted spectral absorption of the blue *Velella* pigment in the red and violet regions, but discerned no distinct bands. They also state that the blue colour gives way to red in mineral acids, violet in alkali, and turns reddish brown on drying, heating, or treatment with alcohol or toluene. Kropp's observations on the blue pigment in both *Velella spirans* and *Fiona marina*, the nudibranch which devours this siphonophore, agreed in many ways with the findings of Haurowitz & Waelsch. They noted, however, that suspensions of the pigment from either source in distilled water showed a region of absorption in the red, between 655 and 685 mμ, without sharp bands, and exhibited reddish opalescence by reflected light. The pigment was chemically unstable, yellow at neutrality, pink to red in acid, and yielded, on treatment with alcohol, a white flocculent precipitate and a red solution. Observations by Dr J. H. C. Smith (personal communication) on a similar blue coelenterate yielded results like those obtained by Haurowitz & Waelsch and by Kropp, in that treatment with alcohol produced a change of the blue colour to orange or red.

Such blue pigments are classified herein among 'miscellaneous pigments' pending further investigation. We agree with Verne (1930) that they are all probably carotenoid-protein conjugation compounds, similar to those encountered in numerous blue,

green or purple crustaceans (Kuhn & Lederer, 1933; Kuhn & Sörensen, 1938), the blue or purple sea-star *Pisaster giganteus* (Fox & Scheer, 1941), the grey 'sand-crab' *Emerita analoga*, the spines of the soft star *Astrometus sertulifera*, and the skin of the purple nudibranch *Flabellina iodina* (unpublished experiments, D. L. Fox).

We know less about the red-brown pigments. Many Scyphozoa are decorated with these or other coloured pigments instead of blue (Mayer, 1910). Sometimes as in *Cyanea capillata* the colour is blue or brown, according to the variety. Chemically these brown pigments seem to resemble blue ones, and investigation may also show that they are carotenoid-protein conjugation compounds. M'Kendrick (1881) and MacMunn (1890) obtained a brown pigment from the scyphozoan *Chrysaora hysocella*. In its granular condition in the animal and in some of its properties it resembles the blue pigment of *Rhizostoma*. In a brief note Griffiths & Platt (1895) describe a violet pigment which they term 'pelagein' extracted with the lipoids from a scyphozoan, *Pelagia* sp. From their account of its solubility in organic solvents it resembles a carotenoid, but it was said to possess no characteristic absorption bands. They assigned to it an empirical formula containing nitrogen without giving any details of their chemical procedure. Whether or not these red-brown and blue pigments are truly related and are shown to be carotenoid proteins, there seems no doubt that a number of pelagic coelenterates have independently developed similar colouring matters. Pigments of this class may also occur in other phyla. M'Kendrick (1881) suggested that owing to its similar properties the blue colour of the ciliate *Stentor caeruleus* (Lankester, 1873) is related to the blue pigments of the pelagic coelenterates. It is of interest that Emerson (1929) records a red pigment from another ciliate, *Blepharisma* sp., with a three-banded absorption spectrum recalling that of the *Stentor* pigment, but with the bands situated in the short-wave end of the spectrum.

Further work may show that the substances we have discussed above belong to well-known classes. This does not seem to be the case with a well-defined series of pigments found in the skeletons of the Alcyonaria. Krukenberg (1886) originally classed them with the lipochromes as 'rhodophans', perhaps combined with calcium. But most of the latter have proved to be carotenoids, and the alcyonarian skeletal colours differ from these in their properties. In the first place they are much more stable. The lasting nature of the red pigments of the precious coral *Corallium rubrum*, of *Tubipora musica*, and of the reds, oranges and yellows of many other alcyonarians is well known. This is true of the dried corals and of museum specimens preserved in alcohol. The uniquely blue pigment of *Heliopora caerulea* is more intense in the centre of the corallum than on the exposed surface. This may be due to fading under the influence of light. Bourne (1895) found that the grass-green solution of the pigment in dilute ammonia fades to yellow on prolonged exposure to strong sunlight.

The skeleton of the Alcyonaria differs from that of the great majority of the Madreporaria in that the skeleton itself is usually coloured. The pigments are actually present within the calcareous spicules themselves, which in the Alcyonaria are pervaded by a matrix containing a considerable amount of organic substance. They differ entirely from those of the polyp tissues or of the zooxanthellae which these sometimes contain. The pigments from the tissues are carotenoid in nature and are readily soluble in various organic and other solvents, despite Krukenberg's (1887) difficulty in detecting lipochromes in corals.

Alcyonarian coloration is often due to the spicular colours, but occasionally it results from other pigments such as the carotenoids. The red colour of the common European gorgonian *Eunicella verrucosa* is due to the presence of carotenoid droplets in cells of the coenenchyme. The colour rapidly fades on drying and is slowly extracted by alcohol. The spicules of this species are colourless (Studer, 1914). We shall see that this pigment system differs entirely from that of *Corallium*. On the other hand the eggs and larvae of both the red and white varieties of *Eunicella* have, according to Studer, a very stable pigment which is 'coral red' and not orange-red: perhaps this resembles the spicular pigments of other Alcyonaria.

It is curious that almost all the work on alcyonarian skeletal colours has been done on the blue pigment of *Heliopora caerulea*, an organism which stands apart from the rest of the group. Doubtless the unusual blue coloration of the coral was responsible for the attention it received. The first serious work on the subject was that of Moseley (1876), followed by that of Bourne (1895) and the detailed study of Liversidge (1898). Their accounts are in general agreement. The pigment withstands hot potash and hot 25 % sulphuric acid. It is insoluble in ether, carbon disulphide and cold neutral ethyl alcohol. It dissolves in hot ethyl alcohol or in alcohol which is slightly acid or alkaline. It is readily soluble in formic and glacial acetic acids and to a decreasing extent in higher fatty acids. It is soluble in ammonia and potash. It is insoluble in aqueous solutions of sodium chloride and sodium nitrate, and in hydrochloric acid. Decalcification of the coral by dilute hydrochloric acid frees the pigment in suspension. The pigment is bleached by chlorine and also by reducing agents such as nascent hydrogen. When dried and ignited it leaves a small amount of colourless ash. It is evidently not a carotenoid.

Of the red and yellow colours of the alcyonarian skeleton we know much less. Long ago Vogel (1814) suggested that the colour of the precious

coral *Corallium rubrum* was due to the presence of one part in 88 of ferric oxide. The pigments are in fact organic substances. Nevertheless, the presence of iron is probably significant, as recent work by Durivault (1937) and Ranson & Durivault (1937) showed. Unfortunately, their notes are as brief as they are interesting, and one looks forward to further information in more favourable times.

Durivault found the yellow, garnet-red, red-brown or violet-red pigments of *Alcyonium palmatum*, dredged from the Mediterranean, to be concentrated in the spicules, apparently associated with calcium. Various tests for carotenoids gave completely negative results, and the skeletal pigment failed to dissolve in standard carotenoid solvents. Histochemical tests for carotenoids on decalcified skeletal sections, i.e. with iodine, sulphuric acid and chromic acid, were also negative. The pigment dissolved to some extent in cold potash, and was destroyed by treatment with concentrated hydrochloric, nitric, sulphuric acids or with glacial acetic. When attacked by dilute acids iron could be shown to be released from the dissolving spicules. The iron could be detected by the Prussian blue test and by the thiocyanate reaction. Durivault believes that iron, from the external medium, combines with a calcium organic complex incorporated in the skeletal matrix, and is responsible for the conspicuous colour of this coelenterate. Similar positive tests were obtained on some gorgonians.

Ranson & Durivault reinvestigated the pigment of *Heliopora caerulea* and studied in addition that of *Corallium rubrum* and *Tubipora musica*, both bright red species. In *Heliopora* skeletons no positive tests for carotenoids could be obtained, nor did carotenoid solvents dissolve the blue pigments. The same results were obtained with *Corallium* and *Tubipora*. The skeletons of all three genera yielded positive reactions for iron, and Liversidge had earlier reported phosphate, calcium, magnesium and iron in the ash of *Heliopora*. Ranson & Durivault believe that iron is combined with a calcium organic complex in the skeleton of *Heliopora* just as in *Alcyonium*. They believe that the horny matrix of the spicular skeleton has a special affinity for iron, which combines with the calcium organic complex to give the various pigments characteristic of the group. It will be interesting to learn what kind of pigments these are. In some ways they recall the 'lakes' formed between metals and dyes such as alizarin.

The Alcyonaria are not alone in having 'fast' colours laid down in a calcareous skeleton. A very few madreporarian corals have coloured skeletons, as in some Fungias and certain members of the Eupsammidae (Moseley, 1876), and in *Dendrophyllia nigrescens* (Silliman, appendix to Dana, 1846). The Stylasterina among the hydrocorallines possess many species with fast red or pink skeletons, while in the genus *Distichophora* there are violet, red, orange and brown varieties (Hickson, 1924). We may note also the brilliant and unfading red of the calcareous skeletons of the sessile Foraminifera of the genus *Polytrema*.

Accurate determinations of the iron content of the calcareous skeleton of alcyonarians would be of very great interest. Not only would they throw light on the nature of the pigment but they might provide us with a new line of evidence on the vexed question of whether the palaeozoic tabulate corals are related to the Alcyonaria or not. Clarke & Wheeler (1922) have given a valuable series of analyses of alcyonarian and madreporarian corals, of which a selection are presented below; ferric and aluminium oxides were weighed and determined together in their work. The results are expressed as percentage of total dry weight:

	$(Al+Fe)_2O_3$ %	Organic matter %
Alcyonium carneum	Trace	40·90
Corallium elatior	0·15	3·14
Tubipora purpurea	0·57	2·27
Heliopora caerulea	0·07	1·55
Balanophyllia floridana	0·69	7·35
Acropora cervicornis	0·06	4·32
Oculina diffusa	0·05	2·01
Agaricia purpurea	0·00	1·98

Unfortunately for our present purpose the analyses were apparently made of the whole organism including the soft parts. As might be expected those analyses which included much tissue tend to contain more iron, in both kinds of coral. But in *Tubipora* there is evidently much more iron than can be accounted for in the living tissue as opposed to the skeleton. On the other hand, the mere trace of iron in the *Alcyonium* is interesting in view of Durivault's easily repeatable demonstration of its presence in the spicules of a related species. It should be remembered, however, that iron compounds often have high tinctorial power even when low in iron content, as in the ferrocyanides and the haematins. We must await further analyses.

There is evidence that another class of pigments, the chromolipoids, occurs in the Hydrozoa. Teissier & Volkonsky (1929) studied the calyptoblast hydroids *Serturella gaudichaudi*, *S. gayi*, *Lafoea dumosa*, and *Aglaophenia tubulifera*, and compared the clear green pigments in these species with the yellow, red and blue pigments of the sponge *Clathrina corinacea*. Though the evidence they present is scanty, we believe that it suggests that the *Clathrina* pigments may be related to carotenoids, and that they are not chromolipoids as these authors suppose. The hydroid pigments, on the other hand, were probably true chromolipoid material, as they were insoluble in the usual neutral solvents, took up

vital stains and reduced osmic acid with gradual darkening. The same green pigments were insoluble in water or dilute acids and alkalis, but slightly so in glacial acetic acid. Alkali turned them red-brown, acid dark green. Treatment with formol or sodium bisulphate, or autolysis following death of the colony, cause the pigment to turn brown.

There remain to be mentioned a number of imperfectly known pigments. The yellow 'uranidines' described by Krukenberg (1887) from a number of corals are little more than a name. Sometimes the pigments described do not really belong to the animal from which they were obtained. Symbiotic algae are common among coelenterates, particularly among the Actinozoa. Some of the recorded pigments originate in these plants rather than in the animal host (MacMunn, 1887), as in the 'Antheagrün' of Krukenberg (1881) obtained from *Anemonia sulcata* (= *Anthea cereus*).

Moseley (1873) found a pigment which he called 'actiniochrome' in the red tips of the tentacles of *Tealia felina* (= *Bunodes crassicornis*), though not in the red body of the animal. It was insoluble in all the solvents he used. He did not find it in *Actinia mesembryanthemum* or in *A. rosea* (these are both *Actinia equina*). MacMunn (1885) confirmed this and showed that the same pigment occurred in the tentacles of several other anemones, notably *Anthopleura ballii* (= *Bunodes ballii*) and the violet tentacle tips of *Anemonia sulcata* (= *Anthea cereus*). He found that it was slightly soluble in glycerine. Fulton (1922) concluded that the same pigment occurs in *Actinia bermudensis* and *Condylactis passiflora*. In this he was wrong, for he based his conclusion on similarity of chemical behaviour to a pigment clearly described by MacMunn as actiniohaematin and not as actiniochrome. MacMunn repeatedly differentiated actiniochrome from actiniohaematin: the former never yielded haemochromogen or other haematin derivatives.

II. PYRROLIC PIGMENTS

1. *Haematins*

Whereas the pigments already mentioned still await proper description, we owe to the comprehensive work of MacMunn (1885, 1886) the knowledge that many actiniarians possess haematins and their derivatives. The occurrence of these well-known substances in such simple animals made a great impression upon him. He showed that in many colour variants of *Actinia equina* (= *Actinia mesembryanthemum*), in *Tealia felina* (= *Bunodes crassicornis*), and in other species, there existed a pigment which he called actiniohaematin. Even the white variety of *Metridium senile* (= *Sagartia dianthus*) showed traces of this substance. This pigment yielded a haemochromogen and a haematoporphyrin indistinguishable from those of haemoglobin. Because of this, and because of the capacity of one of the decomposition products of actiniohaematin to undergo reversible oxidation and reduction, MacMunn concluded that the new pigment possesses respiratory function.

It may be significant that in *Anemonia sulcata* which possesses symbiotic algae, Elmhirst & Sharpe (1920) detected no haematins, though MacMunn considered there might be a trace of them in the tentacles (as *Anthea cereus*). Elmhirst & Sharpe (1923) confirmed the presence of haematin in *Tealia felina* (= *crassicornis*), and found the concentration of both this and of lipochromes higher in shore specimens than in those from deep water. MacMunn (1890) found that the red variety of the corallinomorph anemone *Corynactis viridis* contains a pigment related to actiniohaematin. Its spectrum shows it to be distinct, but nevertheless it yields products resembling haemochromogen and 'reduced haematin'. In the green variety the spectrum is dominated by another pigment with an absorption band between 507 and 481 mμ in the living tissue.

Roche (1932, 1936 *a*, *b*) brings strong evidence that 'actiniohaematin' is not in fact a pure substance but a mixture derived from the intracellular respiratory enzyme systems such as occur in all animals. He relates it to the cytochromes and to Keilin's 'free intracellular haematins'. He shows that the spectrum corresponds to a mixture mainly of cytochrome *b* and of parahaematin similar to Keilin's protohaematin. It is much stronger in the muscles than in other tissues and it is best developed in those species of actiniarians which have the best developed musculature, such as *Tealia felina*, *Hormathia* (= *Bunodes*) *coronata*, and *Cereus pedunculatus* (= *Heliactis bellis*), while it is more feebly developed in *Anemonia sulcata*, *Actinia equina*, *Adamsia palliata* and in the ceriantharian *Cerianthus membranaceus*, which have a weaker musculature. A search for haematins in the powerful and continually active muscles of the Scyphozoa does not seem to have been made. They are well suited for investigation. In the phylum as a whole it is to be expected that the haematins will prove to be of direct physiological importance to the animal; but they contribute little to visible coloration.

MacMunn (1886) demonstrated the presence of porphyrins in numerous invertebrates, including corals such as *Flabellum variabile* and *Fungia symmetrica*. Moseley obtained 'polyperythrin' (1877) from a number of madreporarian corals, from species of *Actinia* and *Discosoma*, and from *Cassiopeia* and *Rhizostoma* among the Scyphozoa. This substance was shown by MacMunn (1886), in Moseley's own specimens, to be a haematoporphyrin.

2. *Bile pigments*

In addition to a haematin MacMunn (1885) found a pigment which he identified as biliverdin in *Actinia equina* (= *mesembryanthemum*). This pigment is situated beneath the ectoderm and in the

base of the foot, and should not be confused with the green ectodermal coloration of some varieties of this anemone; the latter has since been shown to be a carotenoid protein and will be referred to later. MacMunn also found biliverdin in the green parts of *Tealia felina* (= *Bunodes crassicornis*). The pigment of *Calliactis* (= *Sagartia*) *parasitica* apparently belongs to this group. This is one of the few cases at present known where the main coloration of a coelenterate is probably not due to pigments related to carotenoids, though carotenoids play a minor part in the colour pattern even here (Abeloos & Teissier, 1926). The colour of *Calliactis* varies in individuals from light brown to dark red or even violet. This is chiefly due to the presence in the column of two coloured substances. MacMunn (1885) describes them as brown and purple-red. Subsequently Abeloos & Teissier (1926) described them as red granules found in the superficial tissue, with violet granules in the deeper layers. MacMunn considered that these two substances corresponded to a peculiar new pigment he extracted from the ectoderm, the properties of which he described, and to etiniohaematin, the presence of which he demonstrated in extracts of the endoderm alone. But it is difficult to see how the haematins of the pale endodermal tissues of *Calliactis* can be responsible for any of the granular coloration, and there appears to be another explanation for the two colours.

Abeloos & Teissier found that both red and violet granules were due to the same pigment in different forms. Lederer, Teissier & Huttrer (1940) extended their studies, and have isolated this interesting substance, which they have termed 'calliactine'. Their description of its properties agrees in essentials with that given by MacMunn for his new pigment. The authors found it to be a natural reversible *p*H indicator, acids causing it to assume a yellow-brown colour, passing to golden, then orange, while in alkalis it changes to blue, violet and finally red. It is insoluble in ether or chloroform, but soluble in alcohols and in aqueous systems. In 1 % ammonia its absorption spectrum shows a single, rather symmetrical maximum at about 550 mμ, and in 0·5 % hydrochloric acid a curve of similar shape with maximal absorption in the violet at 450 mμ.

Lederer and his colleagues treated alcoholic solutions of the pigment, slightly acidified with acetic acid, with just sufficient dilute ammonia or potash to turn the colour to blue, whereupon the calliactine was precipitated in the form of rosettes of blue-violet needles. This preparation did not melt until the temperature was raised to 300° C. Its empirical formula is given as $C_{22}H_{22}O_5N_4$ or $C_{21}H_{20}O_5N_4$. It shows an interesting series of colours with minor changes in *p*H, and exhibits, in dilute acid, a yellow-orange fluorescence which disappears in alkali. Numerous physical and chemical reactions lead the writers to conclude that calliactine and its derivatives are related to the bile pigments.

But, in addition to this, MacMunn's original experiments brought to light a most interesting property of this substance. It is easily and reversibly oxidized and reduced. An ammoniacal solution is purple when oxidized and yellow-red when reduced. Contact with air suffices to oxidize it when reduced in solution with ammonium sulphide. Whether the differences of the pigment in the tissues are to be ascribed to differences of *p*H, or to different states of oxidation, or to some other causes is not known. But the possibility that we have here a system of functional importance to the animal is clear. The only pigment which recalls calliactine is the purple substance described by Krukenberg (1882*b*) from *Cerianthus membranaceus*. It is insoluble in lipochrome solvents and weak acids, but soluble in ammonium hydroxide. But MacMunn points out that its spectrum is somewhat different, and whether it will prove to be related to the bile pigments remains to be seen.

III. PURINES

We have found no reference to the existence among coelenterates of complex purines such as the pterines; although Chrometzka (1937) points to the wide distribution of these substances in nature. The simplest purines are not true pigments since they are white in pure crystalline form. But in some Actinozoa a brilliant white in some tissues in contrast with colour in other tissues contributes to the colour effect, as in *Sagartia elegans* var. *venusta*. The substance causing this whiteness is generally unknown, but in *Metridium senile* it has been shown to be due to crystalline purines in the tissues.

Mouchet (1929 *a*, *b*, *c*) claimed that the microcrystalline aggregates in the mesenteric filaments of various anemones, including *Metridium marginatum* and *Actinia equina*, were composed of xanthine. He said they were never uric acid, though he based his conclusions only on the relative solubility of the crystals in dilute acids and alkalis, in ammonia and in piperazine hydroxide. Working on *Metridium senile* Fox & Pantin (1941), however, found uric acid, not xanthine, in amounts of 0·15 % of the dry weight. We showed that negligible quantities of guanine were present, and that no measurable amounts of other purines such as adenine, xanthine or hypoxanthine were demonstrable. We concluded that the microcrystalline deposits in tentacles and mesenteric filaments were uric acid, and not xanthine, the occurrence of which Mouchet believed to be general for anemones. In addition to this the yellow sheets of mucous material excreted, even during starvation, from the column and the mouth of *M. senile* were found to contain no carotenoid material, but to yield considerable quantities of uric acid on treatment with uricase and by other methods (Fox & Pantin). Since Mouchet worked upon European species it is probable that his *M. mar-*

ginatum is identical with our *M. senile*. We therefore disagree with Mouchet, but consider it would be dangerous to argue from *M. senile* to other species. In a note Haurowitz & Waelsch (1926) say they found no uric acid in *Actinia equina*, but give no details.

The large white mass of tissue constituting the 'kidney' of siphonophores such as *Velella* is said to contain needles and granules of guanine (Delaunay, 1931). The analyses of Haurowitz & Waelsch (1926) of the tissues of *V. spirans* show the presence of xanthine, hypoxanthine and possibly guanine also.

IV. MELANINS

Despite their wide occurrence in the animal kingdom the only record of true melanins among the coelenterates prior to the studies of Fox & Pantin is a tentative reference by Abeloos & Teissier to the brown pigment sometimes found in the acontia of *Calliactis parasitica*, and to the slight orange colour sometimes seen in the tentacles of this animal. Verne (1926) and Lederer (1940) cite the older work of Krukenberg (1887), who described a group of 'uranidines' encountered in certain corals, sponges and other invertebrates which turn from yellow to black on death of the animal. Verne has suggested that these pigments may be precursors of melanin.

Our studies on the colours of *Metridium senile* have shown that variants containing brown, grey or black tones in tentacles, stomodaeum, ectoderm of scapus, or endodermal tissues contain brown diffuse, or black granular, melanin in various quantities and combinations. We found further that even in red or white variants a complete tyrosine-tyrosinase system is present, and gives rise to melanin on allowing triturated tissues to stand for short intervals. The melanin formation in such ground tissue systems progresses steadily on continued exposure to atmospheric oxygen, through brown chemically reversible stages to the final black, irreversible condition.

V. CAROTENOIDS

As relatively late as 1922 Palmer said 'there is no definite evidence that coelenterates (sea-anemones, corals, jelly-fish and related animals) contain carotenoids, notwithstanding the brilliant colorations which they exhibit'. He gives one reference each to the early studies of Merejkowski & MacMunn on these animals. Though he verges on overstatement when one bears in mind such observations as those contained in Schulze's (1917) classical monograph on *Hydra*, one must agree that the emphasis which he places on the necessity for further study of pigments in this phylum is still desirable.

Such advances as have been made show that carotenoids and their derivatives are probably the main source of colour in coelenterates. MacMunn (1890) pointed out the wide distribution and similarity of the 'lipochromes' in both the plant and animal kingdom. The most important pigments placed in this ill-defined group are the carotenoids, and probably the majority of the red, orange and yellow colours encountered in the Actinozoa are due to the presence of these substances. Merejkowski (1883) had already found 'tetronerythrine' and 'zoonerythrine' in a number of coelenterates, such as *Actinia equina* (= *mesembryanthemum*), *Aiptasia* sp., *Cereactis* sp. and various Hydrozoa. The pigment of *Aiptasia* might be derived from its symbiotic algae, but in some of the others he was probably dealing with astacene or one of its homologues. Recent studies have shown that many of the actinozoan colours are 'animal' carotenoids, not represented in the plant world, although plant carotenoids have been detected in varying quantities even in species which do not possess symbiotic algae.

There is now evidence of the occurrence of carotenoids in almost every branch of the Coelenterata. Lönnberg (1931, 1933, 1934, 1938; and Lönnberg & Hellström, 1932), in his long series of papers dealing with carotenoids in different animals includes in the latter a considerable list from the phylum. He characterizes his pigments chiefly by certain of their chemical properties such as solubility in various solvents, colour reactions with antimony trichloride in chloroform, or with strong mineral acids, etc.; in a number of instances he records the positions of absorption bands. Studnitz (1914) had already brought evidence to show that the orange-red branches of the alcyonarian *Eunicella verrucosa* were coloured by droplets containing carotenoids which were present in the coenenchyme though absent in the polyps except in local traces. Lönnberg shows beyond doubt the existence of pigments related to carotenoids in the alcyonaria *Alcyonium digitatum*, *Stenogorgia rosea*, *Pennatula phosphorea*, and *Funiculina quadrangularis*. In addition, he finds them in the actiniarians *Tealia* (= *Urticina*) *felina*, *Metridium senile* (= *dianthus*), *Sagartia elegans* (= *undata*), *Actinothoe anguicoma* (= *Sagartia viduata*), *A. lacerata* (= *Sagartia lacerata*), *Bolocera tuediae*, *Stomphia coccinea*, *Hormathia* (= *Chondractinia*) *digitata*, *Halcampa chrysanthellum* (= *duodedimcirrhata*), *Protanthea simplex*; in the madreporarian, *Caryophyllia smithi*; and in the ceriantharian, *Cerianthus lloydi*. He also found them among the Scyphozoa in *Lucernaria quadricornis*. Whether the many colours of the varieties of another stauromedusan, *Haliclystus auricularia*, are entirely due to carotenoids is not known. If so, the variety is astonishing, for there are granular pigments of blue, green, olive, yellow, brown, purple and, rarely, pink colour in the ectoderm (Clark, H. J., 1878). Each individual has but one colour present. Possibly these last pigments are related to the series described above in the pelagic Scyphozoa.

Among the Hydrozoa it has long been known that the red polyp heads of the hydroid *Tubularia indivisa* yield a pigment soluble in alcohol, with an absorption spectrum in the blue between 503 and 468 mμ (MacMunn, 1890). Its solubility in chloroform, ether and alcohol, its absorption spectrum and the colour changes in the presence of sulphuric and nitric acids led MacMunn to decide that the pigment was a lipochrome. Lönnberg & Hellström (1932) have since proved the presence of carotenoids in *Tubularia larynx*. Teissier (1925) also found them in *Clava squamata*. But while they are thus certainly present in gymnoblasts, the evidence for their occurrence in calyptoblast hydroids is conflicting. Lönnberg (1931) points out the necessity of using colonies of these which are free of diatoms. He obtained no evidence of their existence in clean *Antennularia antennina*, and Abeloos & Teissier (1926) make no mention of them in species of *Sertularella*, *Aglaophenia*, and *Lafoea*. In contrast with this Payne (1931) states that she found carotenoids in *Antennularia antennina* and *A. ramosa*, and also in species of *Aglaophenia* and *Halecium*. On the other hand, she found no carotenoids, but only flavones, in five members of the Sertulariidae. Calyptoblasts are not strongly coloured, and possibly their carotenoid content varies with the nature of the planktonic food.

The pigments of the species of *Hydra* are of considerable interest. There exist not merely the commonly recognized green and brown forms, but a whole range of varieties, green, colourless, orange to red, brown or black (cf. Lankester, 1882). The nature of these has been fully discussed by Schulze (1917). In *Chlorhydra viridissima* (=*Hydra viridis*) and other members of the genus the green colour is due to the well-known occurrence of a symbiotic *Chlorella*. Some species of the genus *Hydra* can be temporarily infected with symbiotic algae of various kinds, but the effect does not endure, while in *Pelmatohydra oligactis* even temporary infection is impossible (Goetsch, 1924). In these latter species green colour when it occurs is more usually due, or in *Pelmatohydra* is always due, to green food, such as green chironomids.

Basing his work particularly on *Hydra circumcincta* and *Pelmatohydra oligactis*, Schulze showed that where it was not produced by symbionts coloration in hydras was due to the state of nutrition. Starved animals of all species lose their colour. Well-nourished animals become brown or even black. In all cases the pigments are present as granules of various sizes, particularly in the endoderm cells. Whether all the colours are due to carotenoids is not clear from Schulze's work, but this certainly appears to be the case in red varieties. *Hydra circumcincta*, if fed on ostracods or red copepods, assumes an orange-red colour which increases with the richness of the feeding and vice versa. The colour is due to semi-crystalline granules of carotenoid. When fed on red *Daphnia*, which gave no test for carotenoids (one presumes the colour was due to haemoglobin), the hydras became reddish brown and likewise contained no carotenoid.

Schulze argues that the carotenoids are derived directly from the food: the possibility that they are changed into specific 'animal' carotenoids would not seem to be excluded by his experiments. He also shows that on starvation the carotenoid granules are attacked and gradually disappear. Under certain conditions, as in the body of the mother during egg formation, the red pigment may become brown, to become red again later. Schulze suggests that the apparent care taken to store and use carotenoids indicates that these substances play a special role in metabolism: a remarkable statement considering the time when he made it.

Most of the work already discussed does little more than show that carotenoids are present in the animals, or at most that the chief pigment present belongs to this class. In recent years improved technique has enabled us to study the variety of pigments present in one animal or in different colour forms. The first important work of this kind was that of M. & R. Abeloos-Parize (1926). These authors studied the well-known red, brown and green varieties of *Actinia equina*. They found a red or orange pigment in fine granular form in the ectoderm of the body and tentacles, and a green pigment similarly dispersed in the endodermal tissues. From the brown and green animals they recovered an orange carotenoid, and a red one from the red animals. Subsequently Lederer (1933), and Fabre & Lederer (1934), found in this species a new very unstable carotenoid, actinioerythrin, united as an ester in the red variety. The ester is entirely epiphasic (remaining in petroleum ether when washed with methanol), but becomes entirely and irreversibly hypophasic after alkaline hydrolysis. It is an acidic pigment, destroyed very rapidly on treatment of the alkaline salt (from the hydrolysate) with even such weak acids as acetic or carbonic. The natural ester was readily separated on a Tswett column of calcium carbonate; it melted at 75° C., and gave violet solutions in carbon disulphide, with three absorption bands, at 574, 533 and 495 mμ. In absolute ethanol only one band was reported, at 517–518 mμ. In an impure state, even the ester is very easily bleached by oxidation in the air.

The *green* variant of this anemone possesses, according to these writers, also a red-orange carotenoid displaying the properties of xanthophylls and showing in carbon disulphide two absorption bands, at about 504 and 472 mμ. This new carotenoid exists mostly as an ester, bound to protein, thus assuming a green colour. Both colour variants yielded α- and β-carotenes. The clear blue pigment of the 'marginal tentacles' (acrorhagi?) was concluded not to be a lipochrome.

The investigations of Heilbron, Jackson & Jones

(1935) represent the most careful chemical treatment which has so far been applied to studies of the lipochromes of sea anemones. They investigated four species. They did not specify the colour varieties used, but Dr Jackson kindly informs us in a private communication that in *Metridium senile* it was the red form. The following carotenoids were recovered by chromatography, and in certain instances also by crystallization by these investigators:

Actinia equina. (i) Actinioerythrin. This substance, first described by Lederer, was shown by Heilbron and his colleagues to be an epiphasic ester absorbable on calcium carbonate. It crystallized as red-black needles, m.p. 83–85° C., and had the following absorption bands in carbon disulphide: 574, 538 and 497 mμ. In ethyl alcohol there was one broad band with a maximum at 511 mμ. The presence of this ester indicates that Heilbron was using the red variety of this anemone (cf. Lederer, above). (ii) Violerythrin. This new pigment is an 'acid' or more probably enol-keto carotenoid, *derived by hydrolysis of actinioerythrin*, m.p. 191–192° C. It gave a deep blue colour in benzene, bright blue in pyridine and acetone, violet-red in ether and alcohol and purple in carbon disulphide; in the latter solvent its absorption bands were advanced far towards the red as compared with the parent ester, viz. 625, 576, and 540 mμ. (iii) Xanthophyll (probably a taraxanthin ester) with absorption maxima of 502, 472 and 442 mμ in CS_2. (iv) A trace of carotene.

Anemonia sulcata. (i) Chlorophyll *a* (from symbiotic algae; cf. MacMunn (1885) and Elmhirst & Sharpe (1920)). (ii) Carotene, and (iii) a xanthophyll with absorption maxima at 478 and 450 mμ in carbon disulphide. (Both of these also probably from symbiotic algae.) (iv) Sulcatoxanthin. This is a new xanthophyll insoluble in light petroleum, sparingly so in carbon disulphide, but readily in benzene and alcohol; adsorbed on calcium carbonate or sugar. It has absorption maxima in carbon disulphide at 516, 482 and (450) mμ. It shrinks at 110° C., softens at 125° C., melts at 130° C. and is readily destroyed by mild treatment with alkalis. Its empirical formula is probably $C_{40}H_{52}O_8$.

Metridium senile (= *Actinoloba dianthus*) (red variety). (i) The red pigment is doubtless that described by MacMunn (1890) as having an absorption band between 535 and 565 mμ in the living tissue. Heilbron and his colleagues show that it is an epiphasic ester of low melting temperature, non-crystallizable, which upon saponification at room temperatures yields a red insoluble sodium salt. After hydrolysis the free carotenoid acid crystallized from pyridine in deep violet-red prisms giving a m.p. 195–196° C.; both the free acid and its original ester showed a single absorption maximum in carbon disulphide at 495 mμ. Fox & Pantin (1941) compared this pigment with astacene, from which they showed it to be distinct. They named it 'metridene'. (ii) A small amount of another carotenoid which, in the unhydrolysed state, passed through a calcium hydroxide column in petroleum ether. Its absorption maxima in carbon disulphide lay at 501 and 471 mμ. In addition to these pigments Fox & Pantin found astacene itself and various other carotenoids in this species; they will be mentioned later.

Tealia felina. Heilbron's animals were variable in appearance and in pigment yield, which was small on the whole. There was (i) an epiphasic carotenoid adsorbed as a violet-red zone on calcium carbonate, and resembling actinioerythrin. It was recovered in the form of a violet-black wax, m.p. 65–73° C. and absorption maxima in carbon disulphide; 569, 530, 497 mμ. (ii) An orange-red low melting epiphasic ester, giving on hydrolysis an insoluble red sodium salt whose free acid separated from aqueous pyridine in black feathery needles, m.p. 205–208° C. Both ester and free acid showed a single absorption maximum at 500 mμ in carbon disulphide.

The pigment situation encountered in *Tealia* by Heilbron and his colleagues is comparable with the findings of Fox & Moe (1938) who investigated the small anemone *Epiactis prolifera* Verrill from the Pacific coast of North America. This species occurs in red, green or brown colour types, and is reported from the Canadian to the Mexican border. The red or orange-red variant was the form investigated. It extracted carotenoids were entirely epiphasic, consisted of a trace of carotene, possible traces of an esterified xanthophyll and considerable quantities of a red acidic carotenoid ester. The ester and the free acid alike showed a single broad maximum at 500 mμ in carbon disulphide, reminiscent of the corresponding pigment encountered in *Tealia* by the British workers. We do not yet know the underlying factor producing the green colour in the northern variant, but suspect it may be another case of a protein-conjugated carotenoid, since microscopic examination of specimens collected and preserved for some months in formalin by Dr M. W. Johnson, and kindly furnished by him, did not reveal any zooxanthellae. Possession of both the green and red sources of pigmentation may be responsible for the colour of the brown variant.

Valuable as are the careful studies of Heilbron and his colleagues, they still leave the biologist in need of information. We can only surmise from what pigments in the living tissues the carotenoids the examined were derived, and we do not know beyond doubt the colour varieties of some of the anemones used. From the point of view of the biologist wishing to find out the biological significance of the pigments this knowledge is essential. We would urge on all workers in this field the importance of recording the variety of organism from which the pigment is obtained and the source of pigment in the living tissue.

VI. PIGMENTARY BASIS OF COLOUR VARIETIES

If we remain content simply to list the pigments found in an individual coelenterate the main biological problem eludes us. For among these animals we are confronted not only with abundance of pigment but also with an astonishing variety of colour in individuals of the same species. Nor is this variety primarily a question of habitat. In madreporarian corals such as *Porites* and *Pocillopora* (Wood Jones, 1910), in actiniarians (Fox & Pantin), in alcyonarians (Hickson, 1924) and in Stauromedusae

such as *Haliclystus* (Clark, 1878), observers have commented on the way in which numerous colour varieties live side by side under identical conditions. The same is true of pelagic coelenterates, as in the Hydromedusae and the Scyphozoa (Mayer, 1910). Sadly enough this has led to neglect of exact descriptions of pigmentation, for, as Hickson says, colour is considered a 'bad' systematic character. More recently Stephenson has shown the specific importance of range and pattern of coloration.

In some cases variation seems to be of environmental origin. Schulze's work shows that colour variation in *Hydra* depends on nutrition. Elmhirst & Sharpe (1920, 1923), who investigated correlations between the light intensity of the environment and the degree of pigmentation under both natural and artificial conditions, concluded that the anemones *Actinia equina*, *Anemonia sulcata* and *Tealia felina* increase the amount of their pigments, including carotenoids, when in a region of greater light, and vice versa. *Metridium* (=*Actinoloba*) showed no changes of pigmentation under the same conditions. Elmhirst & Sharpe's work showed that in some anemones the pigmentation can vary with external conditions. It is conceivable that in the case of *Anemonia sulcata* their results arose simply from the known variation of the population of symbiotic algae with the light intensity, and hence the change in amounts of algal carotenoids. But this cannot be the case in *Actinia equina* which does not possess these symbionts. Here the work of M. & R. Abeloos-Parize (1926) on the alimentary origin of the carotenoids of the varieties of *Actinia equina* is of great importance. From the brown and the green animals they recovered an orange carotenoid, and a red one from the red animals. Animals raised from the eggs upon a carotenoid-free diet lacked carotenoid pigmentation when grown. Also starved animals regenerated the pharynx and tentacular cycles with only a very feeble carotenoid colouring. Such specimens with carotenoids reduced or absent rapidly recovered the normal pigmentation when fed on a carotenoid-rich diet of shrimps' eggs; furthermore, the regenerating 'browns' and 'greens' reformed their orange pigment, while former 'reds' fed on identical diet recovered their red colour. The work of these investigators indicates two things. First, that in this species the carotenoids are derived ultimately from carotenoids in the diet, and secondly, that there is an important specificity either in the primary selective assimilation of a given pigment, or in the metabolism of a common pigment selected by either variant. Single pure carotenoids added to the diet were not tried.

Like Elmhirst & Sharpe, Fox & Pantin found that the colours of the varieties of *Metridium senile* are much more stable than those of *Actinia equina* seem to be; and though controlled feeding experiments have not been completed, the colour does not appear to change over long periods under a variety of aquarium conditions and of feeding and starvation. Whether this indicates ability in the varieties of *Metridium senile* to construct their carotenoids from simpler substances remains to be seen. But the colour varieties of *Metridium* are certainly stable and maintain themselves side by side in nature apparently on an identical diet, and our analysis showed a complex and surprising set of conditions in them. The varieties prove to be based on a series of biochemical differences of pigmentation which may be classified in four qualitative divisions, viz. (1) white forms, lacking pigment (save for traces in ripe gonads); (2) carotenoid types of pale yellowish, orange, pink or deep orange-red; (3) melanistic types of pale brown, buff, grey, deep brown, or even partly black; and (4) mixtures of (2) and (3). White forms seem to be the most common, with reds a fairly close second, but at some stations the relative order of abundance is reversed. Perhaps to these should be added a bright yellow variety only rarely observed by naturalists on European coasts and not seen by us. Guberlet (1936) reports a yellow form of *M. marginatum* (=*M. senile*?) from deeper water in the north-western coast of the United States.

The substance of a table summarizing the relative abundance and class of carotenoids encountered in various typical colour groups is reproduced below from Fox & Pantin. The chief carotenoids are italicized. For the sake of greater completeness, the relative development of melanin is also recorded. All colour types possess the complete precursor system for melanin formation, the whites and reds resemble cases of 'dominant albinism' (Gortner, 1911).

Colour	Melanins	Carotenoid
White	Little or none	Very little: *Astaxanthin esters* and free astaxanthin
Brown (various shades)	Varying degree	Least. Astaxanthin esters, or metridene esters, carotenes, xanthophylls, xanthophyll esters
Yellow-orange	Little or none	Considerable. *Metridene esters, xanthophyll esters*, carotenes, xanthophylls
Red with brown	Varying degrees	Much. *Metridene esters*, or astaxanthin esters
Red	None	Much. *Metridene esters*, sometimes accompanied by free or esterified astaxanthin, free metridene, xanthophylls, carotenes

In the light of the discovery by Kuhn & Sörensen (1938) that astacene is a chemical artifact of the naturally occurring dihydroxy-diketo-β-carotene, we have recorded the presence of *astaxanthin* esters in animals from which we recovered *astacene* in the pigment hydrolysates.

Of the white animals analysed, small quantities of esterified, and still lesser quantities of free astaxan-

thin (i.e. yielding free astacene on treatment with alcoholic potash) were the only carotenoids recognized. Red animals contained from 8 to 10 times as much pigment (in 'astacene equivalents' as measured photometrically) as did the white variety. In the red, furthermore, the carotenoid material was chiefly esterified metridene. β-carotene and astaxanthin esters were usually absent or in minor concentrations, while unesterified metridene (or possibly hypophasic esters), astaxanthin, other relatively heavily oxygenated xanthophylls, and also small amounts of a xanthophyll resembling lutein, were occasionally encountered. Preliminary experiments failed to reveal an oxidase in the white variant capable of destroying the pigment, metridene, from the red form, but other carotenoids were not tested in this way.

Yellow-orange animals showed mixtures of carotenoids, wherein xanthophylls resembling zeaxanthin and lutein, free and esterified, accompanied β-carotene and metridene esters. Esterified zeaxanthin and lutein seemed at least as plentiful as metridene in some such animals. In one large yellow-orange specimen much free zeaxanthin was encountered. Red-brown animals were fairly variable. Some contained esterified and free metridene, accompanied not by free xanthophylls or carotenes but by an esterified xanthophyll with a spectrum resembling that of pectenoxanthin. Another large specimen yielded only small traces of free metridene in the hypophase, possessed no carotenes, ordinary xanthophylls or their esters, but considerable quantities of esterified astaxanthin.

The members of the melanistic (brown or grey) series contained the lowest concentrations of carotenoids (often less than whites), but exhibited the greatest variability in kinds and combinations of these pigments. Some brown, or brown and grey, specimens contained astaxanthin, a fact which was found to be correlated with the possession of pinkish gonads. If the gonads were unripe, astaxanthin esters were lacking, even though other carotenoids might be present. Certain specimens in a sexually unripe condition were found to contain no carotenoids excepting traces of xanthophyll esters, while others might yield small quantities of a carotene, xanthophylls and their esters, and another carotenoid which became acidic on hydrolysis (probably astaxanthin or metridene). Among the carotenoids separated we encountered fractions with absorption spectra agreeing closely with that of β-carotene, other fractions very similar to the lutein or pentaxanthin class, and one which when hydrolysed became hypophasic and possessed a single narrow band in carbon disulphide with maximal absorption at 489–490 mμ.

Our work on these anemones shows how complex the pigment situation can be in each indivudual animal. Several distinct carotenoids may be present in addition to other pigments. Each variant may have its own set of pigments as well as subtle biochemical differences from other varieties.

VII. BIOCHEMICAL SIGNIFICANCE OF THE PIGMENTS

Fox & Pantin (1941) discussed the significance of the pigments in *Metridium*. This has two aspects: the adaptive value of the coloration of the organism in relation to its environment, and the part played by the process concerned with pigment formation in the biochemistry of the organisms. With regard to the first of these we concluded that the colours were not adaptive. This seems to be true not only of many sea anemones but of many marine invertebrates of all kinds (M'Intosh, 1901). The biochemical significance of the pigment-forming processes is still obscure even in the case of the carotenoids, the pigments about which we know most. Merejkowski (1881) considered that the instability of lipochromes in light and in air indicated that they served the animal as respiratory aids. MacMunn (1890) said there was no good evidence for this, and this is still true. Nevertheless, the idea was revived by Studer (1914) to account for the common association which he showed between the white variety of *Eunicella verrucosa* and the presence of symbiotic algae: in the white form he supposed respiration was helped by the algae, in the red form by use of the pigment. But one is tempted to relate the abundance of the algae to the relative absence of light absorption in the white variety. There does appear to be some relation between pigment development and light intensity. Studer himself drew attention to the fact that red varieties of *Hydra* show an increase in carotenoid pigmentation in situations of high light intensity, and we have already mentioned Elmhirst & Sharpe's observations that the pigmentation of some species of anemone is less in dim situations, but some of these possess no algal symbionts so that the effect does not necessarily depend on their presence.

Schulze's observations on *Hydra* and those of the Abeloos-Parizes on *Actinia* show the dependence of the carotenoids on the food supply. It might be supposed that these were simply taken up passively by tissues containing fatty material. We ourselves noted that in pale variants of *Metridium* the small quantities of carotenoids actually present are stored for the most part in the gonads. But the Abeloos-Parizes' work also shows that absorbed carotenoids may undergo change, so that absorption is not entirely a simple process. Further, Schulze found that there was active transport of carotenoid in *Hydra circumcincta* during regeneration and gamete formation. Carotenoid material streams into the maturing egg, where it accumulates as fine granules, not simply dissolved in the yolk. This transport leaves the parent deficient in pigment. This process does not take place in all species; in *Pelmatohydra oligactis* (= *Hydra fusca*) the gonads are not red.

Transference of carotenoid material may be important for development. Teissier (1925) found in the eggs of *Clava squamata* and other Hydrozoa a carotenoid-protein complex which decomposed during early development, releasing free carotenoid. Emerson & Fox (1940) draw attention to the general accumulation of carotenoids in reproductive structures of animals and plants, and to the specific part they have in some cases been shown to play in the fusion of gametes. These authors also found that in the aquatic fungus *Allomyces* the male reproductive cells accumulated carotene, while the female cells remained uncoloured. There is an isolated observation in the Coelenterata which may parallel this. In 1901 M'Intosh stated that the ovaries of *Aurelia flavidula* (=*A. aurita*) are yellow, while the testes are red. Guberlet (1936) makes the same statement, based apparently on her own observations. We have not yet had an opportunity to investigate the chemical side of the sexual differences in pigmentation. The importance of carotenoids for regeneration is shown in Schulze's work in that regeneration in *Hydra* is accompanied by active movement of carotenoid through the tissues. In addition, Okada (1927) demonstrated that the part of the stalk tissue of *Corymorpha tomoensis* which is rich in red pigment is also tissue which has special power of regeneration.

Little as we know of the function of the carotenoids in any individual, we know even less of the significance of varieties containing different pigments. At first glance it is tempting to assume that the presence or absence of pigments such as the carotenoids in the varieties of a coelenterate may depend merely on their respective capacity to store, or to oxidize and destroy these very labile compounds. Thus we might expect a tendency in purely melanistic forms which produce melanin by an oxidative attack on tyrosine, to exclude storage of the easily oxidized carotenoids. In *Metridium* there is in fact some ground for this expectation because, on the whole, in those varieties of *Metridium* which are richest in carotenoids melanin formation is less pronounced. On the other hand, this distinction is not absolute, and some varieties of *Metridium* store both the oxidation product melanin and the carotenoid pigments. But it is factors of this sort which determine the colour of varieties among some plants, the nearest approach to the colour varieties of coelenterates (Scott-Moncrieff, 1937).

Whatever causes pigment to accumulate, the pattern of its deposition is closely related to structural differentiation throughout the Coelenterata. Stephenson (1928) has pointed out the intimate correlation of the complex and beautiful patterns of sea anemones with the underlying structure in them. We may cite as examples the relation of the red pigment on the disk of *Tealia felina* var. *coriacea* to the endocoelic spaces round the base of the tentacles, or the accumulation of endodermal melanin along the mesenteric insertions of some varieties of *Metridium senile*. In different varieties of anemones, structurally differentiated regions may be picked out in this way, sometimes by one colour, sometimes by another. Such a relation between organization and pigmentation is common throughout the coelenterates and many other groups of animals (Tylor, 1886). It is particularly evident in marine organisms such as the brightly coloured Polychaeta. Among these Allen (1927) has shown a peculiar relation between autotomy and pigmentation in *Autolytus macrophthalma*. The worm has a series of natural breaking points. These occur in a regular manner every few segments, and their position is marked by the absence of yellow pigment in the septa.

Pattern of pigmentation is not due solely to the casual accumulation of a particular pigment in a particular kind of cell. This is most evident when we compare colour varieties. Thus, the red variety of *Metridium* usually contains carotenoid throughout the whole column, but Fox & Pantin record examples in which the pigment is restricted to the scapus and again others in which it is restricted to the capitulum. Each particular tissue has its own colour processes, and the same tissue is linked with different processes in different varieties.

Throughout the Coelenterata great variation of pigment pattern is common. Apart from the Actinozoa we may call to mind varied red, yellow and brown patterns among Scyphozoa such as *Cyanea capillata* and *Cassiopeia* spp., or the varieties seen in Hydromedusae such as *Turris pileata* (Mayer, 1910). As Stephenson says of anemones, it is the plan of the pattern which is characteristic of the species rather than the particular coloration.

VIII. GENETIC SIGNIFICANCE OF COLOUR VARIETIES

The origin of such variations of pigment pattern is a genetical question on which no work seems to have been done. In many coelenterates the colour varieties, although connected by intermediates, centre round several distinct types and are thus true cases of polymorphism (Huxley, 1942). The condition in *Metridium* is typical of many. Fox & Pantin imply that since the colour patterns of *Metridium* seem to be non-adaptive they might be due to unselected mutations. The system of coloration in *Metridium* shows a rather striking parallel to the system of the human blood groups. The latter depends on a few simple genetic differences (Wright, 1940). Colour differences in coelenterates recall colour differences in flowers, and like these might be due to simple genetic differences (cf. Scott-Moncrieff, 1937). In both the anemone colour system and the blood group system there is no obvious functional advantage of one variant over another. In both systems several variants are maintained in fairly large proportion, but the proportions vary in isolated communities. Wright discusses this isolation

in connexion with the blood groups. In coelenterates there are many causes which favour it. Not uncommonly in this phylum the effective breeding individual is an asexually produced clone which, barring geological accident, can live on the same site indefinitely. As in *Metridium*, an entire ecological situation may be occupied by the asexual products of a few animals, and the chance of any of the immense number of sexually produced larvae obtaining a foothold in an already occupied site is remote. In the case of the blood groups Wright argues that the system results from random local differentiation. The same argument may apply to the colour systems of organisms like *Metridium*.

However, there is doubt whether unselected mutation will suffice to give polymorphism. Ford (1934) points out that there is evidence that the coexistence of several varieties can apparently only be maintained if this condition offers a selective advantage. Whether the advantage is directly concerned with the possession of colour or with some other indirect effect on the organism, the balance of advantage between a gene and its allelomorphs must be extraordinarily exact if there is to be no selection. Unfavoured by natural selection the rate of diffusion of a gene through a population may be far too slow to account for any observed cases of polymorphism (Ford, 1940). Diffusion would be especially slow in anemone polymorphism because the interval between generations may be so great.

There is no direct evidence as to the nature of any advantage conferred by colour polymorphism in coelenterates. Ford (1934), Fisher (1930) and Huxley (1942) discuss cases of polymorphism which seem to resemble that of *Metridium*; the shell colour of *Cepaea* (=*Helix*) *hortensis* and *nemoralis* (Diver, 1939) is an example. In such cases the polymorphs show dominance to a universal recessive. They appear as heterozygotes, for these have an advantage over the homozygote because the genes concerned are closely linked with lethals, so that the homozygotes are less viable. If the polymorphs of *Metridium* compare with these, we might expect that the common white variety was a universal recessive and not a dominant albino as Fox & Pantin (1941) earlier suggested. Breeding experiments to determine this would be interesting. Whatever the genetical mechanism of such colour polymorphism (cf. Haldane, 1930), it should be remembered that it is very common among invertebrate organisms. One may refer to colour varieties which live side by side in sponges such as *Halichondria panicea*, in Turbellaria such as *Polycelis nigra*, in nemertine worms such as *Lineus ruber*, as well as in various molluscs.

The genetical problem of the colour varieties of coelenterates may be stated as follows. As exemplified in *Metridium* the varieties seem to be based on the intermixture of a few simple pigment systems in different proportions. The resulting varieties live side by side without obvious relative advantage to one or other. The proportions of the varieties vary, but most are well represented on any one ecological site. The animals are peculiar because the prevalence of asexual reproduction often means that the effective breeding individuals are few and of immense longevity; while the sexually produced larvae occur regularly in immense numbers with only a remote hope of settling. Any hypothesis put forward must take account of the widespread character of colour polymorphism among the simpler invertebrates: special hypotheses to meet particular cases are therefore improbable.

IX. SUMMARY

(1) The nature, variety and significance of coelenterate pigments is discussed. They may be grouped under: miscellaneous pigments, the pyrrolic series, purines, melanins, carotenoids. (2) The nature of several well-known pigmentations is not fully understood. Chief of these are the deep blues and browns of pelagic coelenterates, possibly due to carotenoid-protein compounds; and the remarkable stable colours of the spicules of Alcyonaria. The latter appear to be combined with calcium and iron. (3) Of the pyrrolic series haematins are common. They play little part in coloration and are probably simply part of the intracellular respiratory system. Bile pigments also occur and attention is drawn to the interesting pigment of this kind present in *Calliactis parasitica*. (4) Purines only contribute white to the colour pattern of some anemones. They occur as uric acid and possibly as other purines. (5) Black and brown melanins occur in *Metridium senile* and probably in other anemones. Even white anemones may possess a complete tyrosinase system. (6) It seems that coelenterate colours are due chiefly to carotenoids. These are found in species from all parts of the phylum. Though the evidence is incomplete, the carotenoids are in some cases known to be derived from the food (*Hydra*, actinians). Sometimes at least, however, they are altered to 'animal' carotenoids, as in the 'metridene' from *Metridium*. Different colour varieties may form chemically different carotenoids. (7) Detailed study of actinians shows a surprisingly complex pigment situation. An individual may possess several distinct carotenoids, and different varieties of the same species are characterized by different series of pigments. (8) Little is known of the biochemical significance of the pigments. There are indications that the presence of carotenoids is in some cases related to light intensity. There is also evidence that these pigments are of importance in gamete formation and in regeneration. The biochemical significance of the varietal colour differences is not known. Attention is drawn to Stephenson's observations on the importance of range of colour and of colour pattern in relation to morphological axes of differentiation. (9) The genetic significance of the existence of colour varieties is discussed. True polymorphism with respect to colour is common in coelenterates. No genetic explanation can be offered without further experiment. The resemblance of the colour systems of *Metridium* to the blood group systems in man is commented on. Attention is drawn to the widespread character of colour polymorphism in the lower invertebrates.

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Contribution No. 239

FOSSIL PIGMENTS

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FOSSIL PIGMENTS

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Biochemical chapters of the ancient past are suggested by the presence of numerous organic pigments of well-defined properties in natural deposits of thousands of years' standing. Chlorophylls occur throughout the world of green plants, accompanied by the red, orange, or yellow fat-soluble members of the carotenoid series, whose colors are masked in green leaves and in many unicellular algae, but are familiar to all as extravagantly displayed in many flower-parts, fruits, certain autumn-colored leaves, and in some roots, such as the carrot. Many carotenoids are synthesized also by bacteria and fungi. Pigments of the same class are assimilated from food and stored in the skin, liver, fat, milk, eggs, and other tissues or products of innumerable animals. Many carotenoid compounds are present in the vast populations of microscopic plankton organisms of the sea, and are conspicuous also in various tissues of the larger plants and animals found there. It was the impressive display of carotenoids in many obligately carnivorous or detritus-feeding animals of the ocean's unlighted depths, destitute of living photosynthetic plants, which gave the initial impetus to the writer's search for supplies of such pigments in bottom sediments.

In natural deposits of moderately great age, such as those of certain moor soils, muds from lakes, marshes, underground caves, and notably deep-lying sediments of the ocean floor, molecular fragments of chlorophyll and of animal homologues, such as the hemin of hemoglobin, still persist as various porphyrins. These occur side by side with various carotenoids, although the marine carotenoids appear to decrease with age (depth of stratum) just as does total organic matter.

In organic fossils of great ages (e.g., petroleum, shale oils, and coal) porphyrins occur, but are no longer accompanied by carotenoids, these having been long since oxidized, or, under appropriate conditions, perhaps reduced to colorless hydrocarbons.

Thus, progressing from living material to fossil substances of increasing age, complex molecules such as chlorophyll and hemin are the first pigments to undergo degradation, persisting for but a very short time after the death of organisms; carotenoids are represented in anaerobic deposits of great age in a biochemical sense, i.e., thousands of years, antedating even the Pyramids of Egypt; and these intermediary fossils are in turn outlasted by the porphyrin fragments of the original more complex pigments; some of these porphyrins persist into eons of geological time. Various chlorophyll- and hemin-derivatives in bituminous rocks, petroleums, mineral waxes, asphalts, and other fossilized materials have been extensively discussed by others. The present discussion is concerned primarily with the occurrence of carotenoids in sediments recovered from ancient burial in the bottom of the sea.

Let one haul up a solid cylindrical column of mud, collected from the ocean floor with a special coring device, draw off the superficial water, submit the sample preferably to further drying in an evacuated container, and subsequently treat the material repeatedly with appropriate organic solvents such as acetone or alcohol. The filtered extracts show deep green-brown, greenish orange, or yellow colors depending on the proportions and relative concentrations of chlorophyll break-down products and carotenoids present. No unaltered chlorophyll is encountered, but numerous derivatives of it are separable. A number of different carotenoids are always present, including hydrocarbons of the $C_{40}H_{56}$ series, and so-called polyene alcoholic or ketonic derivatives, wherein from one to six or more oxygen atoms have entered the molecular constitution to give $C_{40}H_{56}O$, $C_{40}H_{56}O_2$, etc.

The carotenoids as a class are chemically unstable, their crystals or solutions undergoing ready bleaching by atmospheric oxygen. This destruction is enhanced by the presence of light and by elevated temperatures. Carotenoids are also destroyed by acids and in some cases by alkalies. The natural preservation of such ordinarily labile

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compounds over vast ages of time is less surprising, however, when one considers the very special conditions prevalent in buried strata of the ocean's floor. For there, free oxygen is lacking, light is absent, temperatures perpetually approach 0° C., and approximate neutrality, approaching that of body fluids, persists. And, while native chlorophylls are readily broken down to their still-pigmented residues by ordinary chemical or biochemical processes, carotenoids are altered or absorbed in the digestive tracts of most animals only at low levels of efficiency. Thus they may pass again and again through the alimentary canals of various mud-eating animals in a chemically unaltered state. Even those which are assimilated may be stored in the consumers' tissues in a but slightly modified or unchanged condition. Furthermore, carotenoids are relatively refractory toward the ordinary nonoxidative biochemical processes of such environments.

The ultimate food supply of benthic animals is in the slow, ceaseless rain of organic matter from lighted zones above. The constant manufacture and supply of carotenoids by diatoms, dinoflagellates, and other marine photosynthetic plants and by certain fungi and bacteria, exceeds the power of animals and microorganisms to assimilate or destroy these pigments. Hence certain parts of the sea bottom have become vast reservoirs for such compounds, which are gradually buried under conditions favoring their preservation over centuries of time.

Preliminary quantitative studies have revealed that, while finely suspended marine detritus may contain as much as 50 mg. of total carotenoids per 100 grams dry weight of material, or 165 mg. of carotenoids per 100 grams of ash-free organic matter involved, marine sediments off the coast of southern California yield amounts of the general order of 0.25 mg. per 100 grams of dry mud, or roughly 10 mg. per 100 grams organic matter in such sediments. This mean figure approximates those of investigated sediments lying 106, 650, and 1,096 fathoms beneath the ocean surface, and to mud strata varying in age between about 15 and 2,500 years. The same pigments have been demonstrated in far older deposits as well. The pigments seem to be present at slightly higher concentrations at the top of the mud than in the deeper-lying strata, and this is to be expected, since any processes of decomposition will have been operative longer in the older material.

Quantitative and qualitative variations are encountered. At one station, for example, material collected from the surface of the ocean floor under 106 fathoms of water, yielded 0.17 mg. of carotenoids per 100 grams of dried material; the first, second, and third foot of a vertical core collected there, representing accumulations of not more than 200, 400, and 600 years respectively, yielded 0.24, 0.06, and 0.05 mg. per cent; the fourth foot of this core, representing an estimated maximal age of 800 years, appeared to contain only about 0.03 mg. per cent. On the other hand, sediment collected at a point not far distant, at a depth of 650 fathoms beneath the water surface yielded, from the eight-foot mud-depth of the core (estimated age = 2,500 years) 0.29 mg. per cent of the carotenoid material. One core, collected from the bottom of the Gulf of California at a depth of 364 fathoms, was 17 feet long. A section of this column of mud, cut from the 15- to 16-foot depth, yielded some 0.61 mg. per cent of carotenoids from the 6,000 to 7,000-year-old stratum.

All marine sediment cores yield greenish porphyrin derivatives as well as carotenoids, the more rapidly deposited material in the Gulf of California being substantially richer in both types of molecular fossil. One core of oceanic sediment was remarkable in its pigmentary stratification. While carotenoids, accompanied by greenish porphyrins, were recovered from the 6-inch, 74-inch and 80-inch depths of this sample (representing approximate respective ages of 600, 7,000, and 8,000 years), an intermediate section of the same sample, taken at the 44-inch depth (about 4,000 years old) contained the greenish porphyrin common to all the sections (absorbing light in the red region of the spectrum at about 669 mμ in petroleum ether), but unaccompanied by any carotenoids. Instead, an oily fraction was encountered, containing a yellowish pigment showing blue-green fluorescence, closely similar in this respect, in spectral absorption and in critical

chemical behavior to a pigment obtained from a sample of California crude petroleum, and similar also to a porphyrin from asphalt.

Perhaps equally as arresting as the fact itself of age-long preservation of carotenoids are the qualitatively selective processes which become evident on chemical examination of the constituent pigments. For, while the xanthophyllic or oxygenated type of carotenoid is greatly preponderant over the hydrocarbon or carotene class in all terrestrial and marine green plants, as well as in the vast majority of marine animals, the proportionality has undergone a reversal in the fossil domain. As examples, various seaweeds may contain carotenes in the order of from 5 to 25 per cent of total carotenoids; microscopic dinoflagellate plants such as *Prorocentrum micans,* and diatoms such as the common *Nitzschia closterium,* both of which occur in vast numbers in the sea, have been found to yield only about 10 per cent of their total carotenoids as carotene, the remaining 90 per cent belonging to the oxygenated or xanthophyllic class. Again, the stores of carotenoids in finely suspended marine detritus or "ocean refuse" are approximately 90 per cent xanthophylls. The great majority of marine animals examined to date reveal their capacity to store carotenoids of the xanthophyllic class with a high degree of selectivity. Indeed, numerous fishes, mollusks, and echinoderms, for example, contain xanthophylls or their chemical esters exclusively, rejecting carotenes in their feces or otherwise disposing of these pigments instead of storing them.

Turning back to the sediments, we encounter there proportions of the hydrocarbon carotenoids ranging from 35 to 40 per cent in the topmost layers of mud, to 70, 80 and even nearly 90 per cent in deeper strata. The 2,500-year-old sample, for instance, yielded 83 per cent of its carotenoids as carotenes, and, in the 6,000- to 7,000-year specimen from the long Gulf of California core, carotenes exceeded xanthophylls by a ratio of more than 2 to 1.

Chemical investigations have indicated that the chief member of the carotene class in marine sediments is the common pro-vitamin A compound, beta-carotene, with pigments resembling alpha-carotene in secondary prominence. These are frequently accompanied by other hydrocarbon types of carotenoid.

Among the xanthophylls of marine muds, compounds not readily distinguishable from the common pigment zeaxanthin, encountered notably in yellow Indian corn, are the most common. These sedimentary pigments are probably identical with certain newly described xanthophylls occurring in diatoms and other marine plants. Fucoxanthin, common in kelps and other algae, is abundant in sedimentary material, as are other less common xanthophylls from algae. Sulcatoxanthin, or peridinin, occurring both in some of the microscopic algae and in certain sea-anemones, is another xanthophyll found prominently in sediments.

The general preponderance of carotenes over xanthophylls in long-standing marine deposits constitutes a reversal of the status prevailing in the great majority of marine organisms. This condition in sediments may have its explanation in several potential factors. In the first place, xanthophylls are more readily oxidized and bleached by atmospheric or dissolved oxygen than are carotenes. While this differentiative influence might be operative even in the deeper realms of the ocean's water, it would not apply beneath the surface of the oxygen-free mud. Secondly, the majority of marine animals investigated store chiefly xanthophylls rather than carotenes in their tissues; their storage and partial degradation of polyene alcohols, with fecal rejection of carotenes could contribute substantially to a gradual preponderance of the latter class of carotenoid in bottom sediments. Finally, there are indications that certain kinds of bacteria and allied microorganisms living in marine muds may contribute carotenes of their own synthesis, and that other such flora may be able to effect the chemical reduction of xanthophylls to compounds of decreased oxygen content, or perhaps even to carotenes.

These ancient biochromic compounds, including porphyrins and carotenoids, together with accompanying oil-soluble substances, may be looked upon as diagnostic features in the continued search for biochemical processes operative in the genesis of petroleum and allied natural deposits.—Denis L. Fox.

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BIOCHROMES[1]

THE natural coloring matters of plants and animals are receiving ever-increasing attention in various fields of experimental biology. The present purpose is to refer briefly to the heterogeneous class of biological pigments and to point out fundamental similarities which would appear to justify their collective designation by the title given to this communication.

Professor Sumner[2] has aptly criticized the term "pigment" as a word of relatively loose application. He insists that the word is best limited to its strictly functional sense, but points out that the term arising from a Latin verb meaning "to paint" has been extended by many biologists far beyond its original significance of an artificially imparted color, to include now all naturally colored substances in living systems. It is true that many Greek and Latin words have undergone gradual modification and expansion so that their present significance departs widely from the original limits of meaning.

The present writer, for one, would accept the broad term "pigment" as signifying, in biology, all colored substances, whether their chief functions appear to include those of imparting concealing or advertising colors, or whether they may participate in known or obscure biochemical reactions, or indeed have a known role at all. The word is widely useful, and will undoubtedly persist in general biological parlance.

There is, nevertheless, a valuable point in my colleague's criticism. The term "pigment" is often applied to a substance which is designed to, or merely happens to impart color to something else, whether man-made or naturally occurring. The classes of pigments which interest biologists do not ordinarily include colored inorganic compounds employed in commercial paints or the synthetic dyes of the organic chemist. The histologist's tissue-stains are useful to him only in the artificial differentiation and identification of protoplasmic structures. Concerning natural colored substances, while these may serve occasionally

[1] Contributions from the Scripps Institution of Oceanography, New Series, No. 240.

[2] F. B. Sumner, *Sci. Monthly*, 44: 350, 1937.

to distinguish color-variants and heritable mutations within species, or to identify organs, tissues or biological products in laboratory specimens, here again the primary consideration is the usefulness of color to the investigator. The chemical nature and possible physiological significance of the chromophoric molecule is merely incidental and often quite neglected.

From the standpoint of the biochemist whose interests are concerned with the metabolic significance of natural coloring matters, the designation of these by a discriminating scientific term has long been desirable. In response to an inquiry, Dr. George M. Calhoun,[3] late professor of Greek in the University of California, once suggested to the writer the descriptive and self-explanatory term *biochrome,* with an adjective *biochromatic* (*biochromic* is perhaps preferable), and a collective noun *biochromy.* This word and its derivatives have therefore been employed in this article and elsewhere.[4,5]

Considering the great chemical diversity among the biochromes or natural biological pigments, why should the biochemist feel justified in setting them apart as a class and in giving them a collective name? Let us return to this question in a moment.

In any series of natural classification there will occur overlappings and inexactnesses at the borders of groups. These will occur especially when both chemical and physiological or other biological criteria are involved in the system. As an example, we recognize, under the general heading of *catalysts,* an extended series of very diverse compounds whose common property is that of controlling the occurrence or the rates of various chemical reactions. Excluding now the inorganic and synthetic accelerating (or retarding) reagents of industry and the chemical laboratory, we have still a large and assorted group of biocatalysts, including the numerous enzymes, hormones and vitamins which promote, maintain, restore or otherwise influence diverse physiological functions. The biocatalysts, although necessarily further subdivided into classes, share as common characteristics,

[3] Personal communication, 1936.
[4] D. L. Fox, SCIENCE, 100: 111, 1944.
[5] D. L. Fox, D. M. Updegraff, and G. D. Novelli, *Archives of Biochem.,* 5: 1, 1944.

first, their origin and occurrence in living systems, and secondly, varying degrees of control over the promotion or rate of given biochemical reactions or chains of reactions in the organism.

Returning to the biochromes, these are likewise placed in a subclass of a great and heterogeneous group of colored molecules because, in spite of chemical and physiological differences as great as those existing among the biocatalysts, biochromes also possess two chief characteristics in common, *i.e.*, their origin and occurrence in living organisms and their reflection of a fundamental chemical property, the selective absorption of light waves in the visible spectrum. Some biochromes are also biocatalysts, *e.g.*, chlorophyll, cytochrome, certain flavines and some of the carotenoids.

Reduction in the vibrational frequencies of certain valence electrons, and molecular resonance,[6] visible as color, are evoked by various kinds and degrees of chemical unsaturation. In many instances, unsaturated chromophoric groupings may impart both color and increased reactivity or chemical instability to the same molecule. Such compounds may therefore assume more readily important biochemical roles (*e.g.*, carotenoids, tetrapyrroles, flavines, some pterins and certain quinones) or may constitute representative by-products of special metabolic processes (*e.g.*, bile pigments, melanins, indoles and certain pterins). Color and biochemical activity are, in such instances, two interlocked effects of the same fundamental molecular phenomenon.[7,8]

Our present understanding of the parts played by various biochromic compounds in the metabolic economy of organisms leaves much to be desired, but it is expected that increased study will extend the borders of our knowledge in this field. It is hoped to treat the subject more fully in a review to be published elsewhere.

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Contribution No. 241

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ZOOLOGY.—*A new species of hoplonemertean* (Paranemertes biocellatus) *from the Gulf of Mexico.*[1]

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Among the nemerteans collected on the intertidal sand flats near Biloxi, Miss., were three specimens of an undescribed species of *Paranemertes* Coe. Only five other species of that genus have been previously reported; four of these were found on the Pacific coast of North America and one on the coast of South Africa. This new species presents such morphological deviations from the others that their description will supplement in some degree the available knowledge of nemertean morphology.

Individuals of this new species, which may be known as **Paranemertes biocellatus** resemble those of *P. californica*, found on the Pacific coast, in size and general appearance but differ in having the proboscis sheath nearly as long as the body, in the character of the proboscis armature, in size and shape of ocelli, and in other morphological details. The species also resembles *Amphiporus bioculatus* McIntosh in having a narrow, pointed head and a single pair of ocelli but differs widely in most other characteristics.

DESCRIPTION

Body long, moderately slender, pointed anteriorly and much flattened in intestinal region. Shape and general appearance similar to the published figure of *P. californica* (Coe, 1904, pl. 15, fig. 2). Size when mature 60 to 120 mm or more in length and 2 to 4 mm in width.

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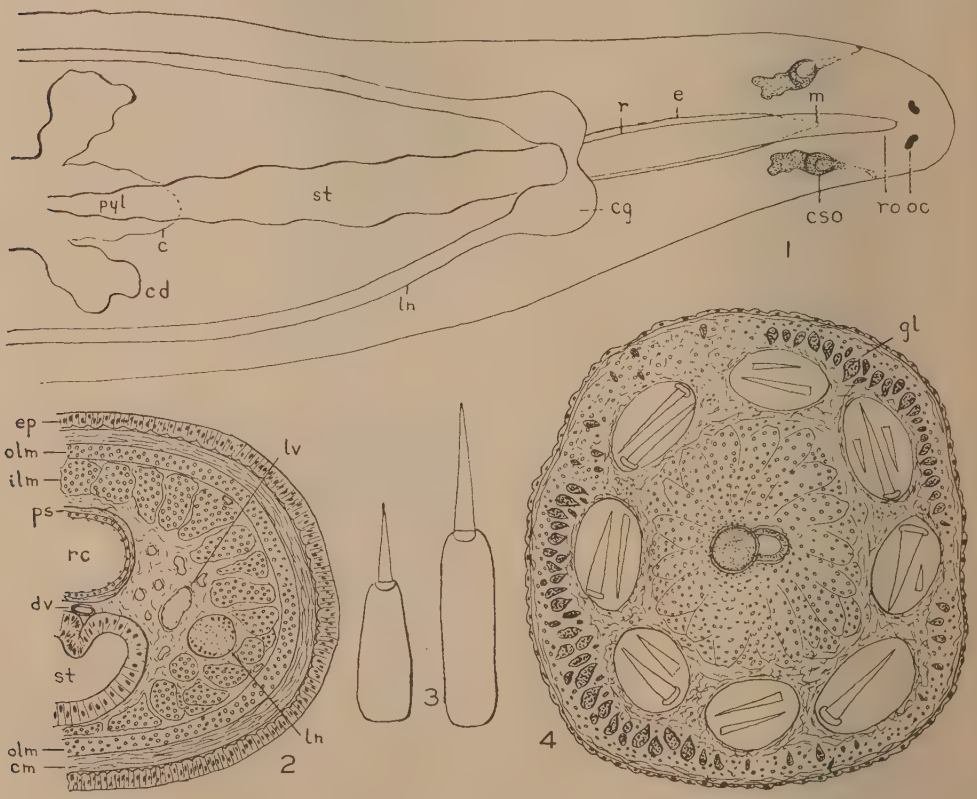
Color.—Although no record is available as to the details of coloration in life, the specimens a short time after preservation were translucent, with a pale opalescence and tinges of green and rose anterior to the intestinal region. The rest of the body varied from pale to deep green, fading to colorless near the posterior extremity. The green color was confined mainly to the intestinal diverticula. The general appearance in life must have been similar to the colored figure of *P. californica* published by Coe (1904, pl. 15, fig. 2), although the colors were presumably paler than those shown in that figure.

After clearing in oil the tissues become pale yellowish with the exception of the intestinal diverticula, which retain their green pigmentation.

Ocelli.—The tip of the head is provided with a single pair of large, kidney-shaped, intensely black ocelli. These are always conspicuous after clearing in oil, and presumably also in life, although they are situated deep in the tissues of the head. In a specimen about 100 mm in length each ocellus measures 0.009 mm in length and 0.006 mm in diameter (Fig. 1).

Proboscis.—This species differs from the other described species of the genus in having



Paranemertes biocellatus, n. sp.: Fig. 1.—Diagram of organ systems in anterior end of body after clearing in oil; *c*, caecum; *cd*, caecal diverticulum; *cg*, cerebral ganglion; *cso*, cerebral sense organ; *e*, esophagus; *ln*, lateral nerve cord; *m*, opening of mouth into rhynchodeum; *oc*, ocellus; *pyl*, pylorus; *r*, rhynchodeum; *ro*, rhynchodeal opening on ventral surface of head; *st*, stomach. Fig. 2.—Portion of transverse section of body posterior to brain, showing the two layers of longitudinal muscles; *cm*, circular muscular layer; *dv*, dorsal blood vessel; *ep*, epithelium of body wall; *ilm*, inner layer of longitudinal muscles; *ln*, lateral nerve cord; *lv*, lateral blood vessel, with branches in surrounding parenchyma; *olm*, outer layer of longitudinal muscles; *ps*, proboscis sheath; *rc*, rhynchocoel; *st*, stomach. Fig. 3.—Central stylet and basis from two individuals. Fig. 4.—Diagram of transverse section through septum of proboscis, showing, in center, stylet basis and canal leading from anterior to posterior proboscis chamber, and longitudinal musculature surrounded by eight pouches of accessory stylets; *gl*, wreath of pigmented gland cells.

the proboscis sheath nearly as long as the body. The proboscis is armed with a slender, nearly cylindrical basis and with four or eight pouches of accessory stylets (Fig. 4). In one specimen the basis was of nearly equal diameter throughout the entire length and truncated posteriorly, while in another specimen it was slightly enlarged posteriorly (Fig. 3). In an individual exceeding 100 mm in length the basis was four times as long as its diameter, measuring 0.24 mm in length and 0.052 to 0.06 mm in diameter. In an individual 45 mm long the basis was only 0.016 mm long and 0.05 mm wide anteriorly and 0.07 mm posteriorly. The stylets are approximately two-thirds as long as the bases. The latter are deep brown in color. There are 12 proboscidial nerves.

Musculatures.—In this species, as in the others of the genus, the longitudinal musculature in the anterior portion of the body is divided into two distinct layers, as described and figured by Coe (1904, 1905) for *P. californica.* Of these, the outer layer is approximately equal to the circular layer in thickness, while the inner layer averages several times as thick. The two layers are separated by a thin sheet of connective tissue carrying numerous blood vessels and branches of the lateral peripheral nerves (Fig. 2). In the brain region this inner musculature closely invests the brain and esophagus and so nearly fills all the space between these organs and the outer muscular walls that the cephalic parenchyma is reduced to small patches. This inner longitudinal musculature is continuous with the proboscis insertion musculature.

In the region of the pylorus the sheet of connective tissue separating the two longitudinal musculatures becomes thicker but diminishes again anterior to the intestinal region, and the two layers become united more posteriorly. The inner portion can, however, be recognized by its larger fibers far back in the intestinal region.

Digestive system.—As shown in Fig. 1, the mouth opens by a long slit into the rhynchodeum some distance posterior to the rhynchodeal opening when the proboscis remains in its normal position within the body. The slender esophagus leads to the elongated stomach and thence to the slender pylorus, which opens into the midgut somewhat farther behind the brain than the distance from brain to tip of head. The caecum is remarkably short and bears but a single pair of diverticula (Fig. 1). The paired midgut diverticula are as in other species.

Blood and nephridial systems.—In the two specimens cut into serial sections the blood vessels were much contracted, both in the head and throughout the body. Near its origin from the cephalic anastomosis of the lateral vessels, the dorsal vessel passes into, but not through, the wall of the proboscis sheath for a short distance and then continues on the ventral side of the sheath to the posterior end of the body. There are numerous connections between the dorsal and lateral vessels.

In neither of the two specimens are the nephridial canals well preserved, nor could the efferent ducts be demonstrated. The same difficulty was encountered in two specimens of *P. californica* (Coe, 1905), although in a third specimen both the canals and the efferent ducts were conspicuous (Coe, 1940).

Nervous system.—The brain is situated farther back from the anterior end of the head than in most hoplonemerteans (Fig. 1). The four ganglia and their dorsal and ventral commissures are of the usual hoplonemertean type. The 12 proboscidial nerves are large and well differentiated from the interneural plexus. The lateral nerves unite posteriorly on the dorsal side of the rectum.

Cerebral sense organs.—These organs, with their sensory and glandular components, are relatively small, elongated structures situated far anterior to the brain. They are connected with the exterior by a pair of ciliated canals leading anterolaterally to the lateral surfaces of the head (Fig. 1).

Reproductive organs.—The gonads are of the usual hoplonemertean type, alternating more or less regularly with the intestinal diverticula. The gametes were not fully ripe in December.

Habitat.—These specimens were found burrowing in intertidal sand flats at Deer Island and at two other localities on the shore at Biloxi, Miss., by M. W. Williams. Cotypes, U.S.N.M. 20641.

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